

ADVANCED BIOMANUFACTURING

A Workshop

Workshop: Advanced Biomanufacturing will identify needs and barriers in the field of biomanufacturing, including research, scale up and implementation, further technological innovation, and regulatory and financial issues. Bringing together leaders in the field to discuss and identify critical issues and challenges interactively, *Workshop: Advanced Biomanufacturing* will assess the current state-of-the-art, the paths to move forward, and identify major barriers.

Holiday Inn Arlington at Ballston, Arlington, VA 22203

Preface

A two day workshop on Advanced Biomanufacturing was held on July 25 and 26, 2013 in Arlington, Virginia. The NSF-sponsored event included participation from many academics, industry representatives and government program managers. This report summarizes the main activities of the event and also provides suggested opportunities and challenges to help move the field of Advanced Biomanufacturing forward. The field is in the early stages of effecting jobs and economic growth, with a bright and important future impact evident based on the rapid scientific advances in recent years and industry progress.

Special thanks are due to Kaiming Ye, Robert Wellek, Ted A. Conway (CBET), and Zhijian Pei (CMMI), NSF, for supporting the event. Similarly, thanks are due to all of those who accepted the invitation to attend and participate in the workshop and special thanks go to the session chairs that took on extra tasks to pre-organize sessions and coordinate the inputs. This group of session chairs also served as the organizing committee for the Workshop and their inputs were instrumental in bringing the appropriate scope and depth to the discussions. The organizing team included: Gang Bao (Georgia Tech), Chris Bettinger (Carnegie Mellon), Cheng Dong (Penn State), Gabor Forgacs (Clarkson), Ali Khademhosseini (Harvard), Wei Sun (Drexel), Chris Voigt (MIT), Peng Yin (Harvard).

Special thanks go to Keleigh Sanford (Tufts) who took care of all of the logistical tasks for the workshop, both before, during and after the two day meeting. Her organizational skills and attention to detail were instrumental in the success of the workshop and to the effective operation and outcomes.

My thanks also to the various government program managers who attended from the NSF, NIH, DOE, FDA, NIST, JDRF, NASA, ONR, and to those of you who gave brief and informative overviews of programs to help engage the participants in terms of program needs, context and opportunities. The positive response and the quality of the talks, the organization of the sessions and the active participation was terrific. I thank all of the participants for their inputs and discussions.

My hope is that this document can serve as a preliminary blueprint to help spur expansion in funding, in new program initiatives, in government support, in industry development and ultimately to have a positive impact on jobs for our students, economic growth and for the long-term sustainability of our planet.

With great appreciation,

David Kaplan, Chairman
Professor & Chair, Department of Biomedical Engineering
Stern Family Professor of Engineering
Tufts University

Summary – A Workshop on Advanced Biomanufacturing was sponsored by the National Science Foundation and held on July 25 and 26, 2013 in Arlington, Virginia. The Workshop brought together leaders in the field, from academics, government and industry to discuss and identify critical issues and challenges related to advanced biomanufacturing. The goals were to assess the state-of-the-art as well as to identify paths forward to bring this field to the impact envisioned, including new research opportunities, new corporate activity, new jobs and a bolster to the US economy. To address these goals, the workshop was run in an interactive and iterative format where session leaders prepared pre-workshop outlines of major topics and then addressed these to the attendees. The workshop was divided into four sessions to reflect the scaling issues that will be important, from the molecular to the systems integration. The vision for the field is to see advanced biomanufacturing emerge as a discipline in academic and industrial communities, as well as a technological opportunity to spur research and industry growth. The vision was refined at the workshop and the product of those discussions is included in this report. To navigate this vision, the paths to move forward and to identify major barriers were a focal point of the discussions. These needs encompass the science and engineering involved, the regulatory and infrastructure needs and the systems integration required. Some of the recommendations, major targets and opportunities were also outlined, including some ‘grand challenges’ to spur interest and more rapid progress in the field. The formation of an initial network in the community has been established. This document summarizes the efforts of the workshop and it is the hope of the participants that it can serve as a guide to next steps in the field for academic, industrial and government needs.

Intellectual Merit - The intellectual merit of the Workshop was in coalescing insight into the field of advanced biomanufacturing in terms of the current scientific state of the art, the technological implications and the future vision for the field. This insight should be a foundation upon which to propel the field ahead in the coming years towards direct impact for the US economy.

Broader Impact - The broader impact of the Workshop is the identification of critical challenges for the scientific and technological communities for this emerging field. Impact on broader educational activities for interdisciplinary needs for students at all levels is also discussed in the report, as well as guidance for the NSF in terms of future directions, insight for government officials at all levels of future needs for growth in the US to support jobs, and broad and new insight into the unique intersections between nature and engineering that remain to be tapped and exploited for a sustainable planet.

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Executive Summary

Definition - *Advanced Biomanufacturing* - The use of biological systems or the products of biological systems to generate new materials and devices, with a view towards scalability and industrialization. These processes include the use of technology to generate biologically-relevant materials and devices wherein biological components and/or processes are included. The key is utilizing building blocks, materials or synthesis systems, such as via cells or related components, to exploit control that biology can provide over materials, from structural hierarchy and complex systems/assembly perspectives.

Specific Aims - The major Aims for the Workshop were to identify the needs and barriers in the field of advanced biomanufacturing with respect to:

- research
- scale up and implementation
- further technological innovation
- regulatory issues
- training/education

Vision - The vision is to advance biomanufacturing as an emerging discipline in academic and industrial communities, as well as a technological opportunity to spur research and industry growth. The anticipation is that this effort will lead to new modes of generating components/building blocks, materials, devices and systems for a range of needs, from medical devices, sensors and devices, to new ways to alter the supply chain, manufacturing environment and environmental compatibility from upfront to downstream components. The goal is to see how this effort can lead to jobs, industry growth and environmentally-compatible synergy. This vision would lead to a return of manufacturing jobs to the US and lead a scientific and technological revolution wherein sunlight, organisms, integrated synthesis and processing and novel biological approaches and ingenuity would provide alternatives to current petrochemically-derived feedstocks and processes.

To navigate to this vision, we assessed where the state-of-the-art is, what the paths are to move forward to reach the vision, and to identify the major barriers. These needs encompass the science and engineering involved, the regulatory and infrastructure needs and the systems integration required. The idea is to disrupt and transform, not to take a small step. However, there are many examples of activities upon which to build, where small successes and opportunities can serve as a guide to the larger impact, eventually leading to a new sector of the economy.

Timing – Why Now?

The scientific tools to support the vision for advanced biomanufacturing have been emerging over the past 10-15 years, empowered by advances in genomics and proteomics, cell biology, process engineering and design and systems integration. These advances, originally focused more on the human genome and needs towards personalized medicine, can now be targeted towards materials, devices, energy and manufacturing in ways not even feasible ten years ago. Importantly, these opportunities are now driven by the many confounding economic and energy challenges presented to society. The opportunity to turn from the ground (oil, gas) to the sky (sunlight) to drive many of these processes, to embrace biological complexity in a new way and to learn from nature in ways not previously considered, can empower the next generation of growth industries while preserving our resources, our environment and supporting human needs.

Historically, advanced biomanufacturing has focused mostly on the pharmaceutical industry, referring to fermentation, purification and formulation needs, including upstream and downstream aspects of the process. This industry continues to thrive and many of the insights and advances from the production of pharmaceuticals can be used as a guide to some of the efforts considered in the workshop.

Important Lessons from Biology

We approached the challenges in advanced biomanufacturing from a hierarchical perspective. This approach was selected in part because this is the model from biology and some emulation of this approach was hypothesized to inform and guide our plans in a positive way. As such, the design and control of building blocks, the ability to program and generate polymers, the concept of self-organizing cells and the ability to print complex tissues and organs provides the scaling to be explored. While this helps to identify the tools, the paths and the systems, it does establish some self-imposed limitations that need to be recognized. Perhaps the largest of these is that in nature these individual scales are not segregated but intimately connected, providing seamless integration to permit efficient and productive systems to function. We will need to keep this in mind as the themes and outcomes from this Workshop are acted upon.

Building Blocks, Molecular Recognition and Hierarchy - The ability to design biological manufacturing processes by encoding information content from the building block stage is one of the remarkable and empowering features in biology, and is also very distinct from current modes of manufacturing in industry. These rules of control (e.g., stereochemistry/chirality, self-assembly, self-sorting, etc.) provide the core of many of the systems discussed. Further, encoding information content at the building block state facilitates the scaling and assembly required to achieve more complex structures and functions in systems. This is not a trivial issue to embrace, as the subtle rules that guide structural hierarchy remain somewhat elusive. However, the general knowledge that small forces (e.g., van der Waal, electrostatic, hydrogen bonds), a role for water in the process of controlling interactions among components, self-assembly in terms of molecular recognition and interfaces, and scaling driven by the sequence chemistry encoded in building blocks (e.g., sugars, amino acids, DNA), can all be exploited in advanced biomanufacturing. Further, at the lower scale, modes to design and synthesize designer building blocks (whether native or nonnative) are in hand, and the modes to encode patterning and spatial features are somewhat in hand but need further robustness and insight. Subtle forces, such as membrane potential and associated biophysical factors are only just emerging as important themes to be exploited in the field.

Bottom Up vs Top Down – A top down approach can be contrasted with the bottom up approach above. For example, building or assembling tissues and structures from the top down remains early stage, yet advances are emerging, from inkjet printing to self-assembling gels to generate complex tissue assemblies. All of these advances inform our enthusiasm to the future potential and suggest that the tools and the basic insights are emerging. Thus, the timing is right to build on these starting points to examine a path forward to advanced biomanufacturing and to empower growth of this field.

Tools - Molecular Biology - Major advances in genomics, proteomics and synthetic biology, with the associated databases and tools, have pushed the ability to design and implement new genetic approaches. These tools empower biological design, control and production processes to a level not previously achievable. While the bulk of this focus has been on *Escherichia coli*, Chinese Hamster Ovary (CHO) cells and a few other systems, the opportunities to expand the repertoire of suitable host systems with scalable options and robust features is now available.

Tools – Polymers - Major advances in polymer synthesis have been driven by insights from the bacterially-derived polyhydroxyalkanoates and efforts to generate bioplastics like this over the past 20 years. Additional insights have come from understanding the cellular machinery required to optimize

polymer yield. Understanding upstream and downstream design needs, tailoring polymer composition and generating useful products from biological systems have all expanded over the past twenty years. Specific insight and studies into the synthesis of polymers, such as alginates, xanthans, hyaluronic acids, amylose/amylopectin, tropoelastin, collagens and others have also helped to push these technologies ahead. Issues of purification, processing, control of polymer features, all feed into these topics. Additional topics, such as purification related to endotoxins is also pursued related to the medical utility for the systems.

Tools – Patterning and Control - Major advances in cell patterning and control would empower a next generation of tissues, devices and systems. These features emerge from developmental biology and require subtle insight and control into stem cell biology, matrix interactions, mechanical forces, electrical forces and many other factors. Self-sorting based on cell receptors and programmed cell functions are endemic to these features. When these aspects of pattern control are then integrated with external manipulation of such features, such as by novel processing tools and deposition processes (e.g., ink jet printing), new generations of complex systems can be envisioned. For example, tissue and organ printing, pattern control of cell biology and many related themes have emerged in recent years.

Tools – Systems Integration - Building devices and the associated manufacturing needs are a critical component of the overall processes above. Without these processes fully integrated into the upstream synthesis and formation of components, the systems will not emerge. Thus, it is critical to consider how we can exploit current manufacturing processes in new ways, how biology solves these processes in a supply/demand way and at scales matched to system requirements, and how energy conservation and recycling/reuse are the routine and not the exception.

Green Technology, Ambient, Water – Advanced biomanufacturing offers a green template for production processes, due to the biologically-derived building blocks as well as the inherent aqueous based processing of materials in nature. Such a foundation has the potential to transform the way many products are made, in concert with environmental compatibility and avoiding negative impacts on the ecosystem. Since all biologically derived materials are inherently degradable, this also provides a template for future resource recycling and reuse and reduced burdens on disposal problems in the environment. This approach also avoids the generation of toxic compounds that are counterproductive in the environment and to human health. The approaches are not without concerns, however, such as the large dependence on water in production and processing, thus, suitable recycling systems matched to advanced biomanufacturing needs will be needed.

Overview of Technical Details and Needs - For the four main sessions covered at the workshop, the following outline of inputs was utilized to craft the main body of this report:

State of the art – where is the field today in terms of science, technology, manufacturing and related issues

Gaps – what are the major missing pieces of knowledge or fundamental understanding that would allow progress in the field and to bring the field to the stage of manufacturing impact

Barriers – what approaches or advances in the field are needed to allow progress and what would these advances allow us to do now that we cannot accomplish today

Art of the possible – if we can address the barriers, what is possible in the field, how would manufacturing practices be transformed in the future

Modeling and simulation – what are the tools needed

Regulatory and costs – what needs to be considered in moving forward in the field

Training & Training – how do we prepare the next generation workforce of researchers, engineers and technicians to support the field of advanced biomanufacturing

Major Recommendations

Global Concepts and Grand Challenges – ‘Bioindustry Challenges’

Some global themes and challenges emerged from the workshop that can be highlighted as ‘generic opportunities’ to spur innovation and ideas in the field of **Advanced Biomanufacturing** or the related topic of **Industrialized Biology**. These concepts can be equated to the Gates Global Challenge approach, although here the themes are directed to growth in the science and industry of Advanced Biomanufacturing. Brief topics and descriptions are provided to help with this type of initiative. This also should be a living list, with new ideas added regularly as appropriate.

These challenges represent the ‘*Major Recommendations*’ as themes that can serve as nuclei for initiatives to fill gaps that permeate the field of Advanced Manufacturing to help spur continued growth. More specific needs are identified in each of the four sessions that were held, as well to provide refined views of needs in the field.

Challenges – Building Blocks

Challenge: Core Bio-Supply Houses and Bio-Factories – This concept is based on the success of oligonucleotide/gene synthesis and supply companies as a model from molecular biology field. Core industries that can supply building blocks for the field would be beneficial, reduce duplication of effort, provide quality control related to future FDA requirements, and provide a growth industry for jobs and infrastructure. Specific targeted products from such industries could include oligonucleotides/genes, purified recombinant proteins, engineered cells, modeling tools, educational software/tutorials/online learning.

Challenge: Synthetic cells to Generate Product ‘x’ – The concept is derived from success with generating synthetic cells, where we envision corporate entities centered on generating synthetic cells with minimal genetic requirements for basic functions. These functional biological ‘shells’ or ‘containers’ – will be available to labs to either order or add genetic machinery to produce specific building blocks of interest. This would improve efficiency of production of building blocks, avoid duplication of effort and streamline eventual applications. For example, cells that can be used to generate specific polymers, such as the components of wood or bone or complex material gradients, would provide a useful template.

Challenge: Pre-programmed Cell Factories to Build ‘x’ – The goal is to genetically pre-program cells to produce different components in an orchestrated approach towards the formation of complex structures. The origins are the need for generating products in resource-limited locations to reduce shipping/logistics burdens and to preserve the environment (for example, in future space travel scenarios where it is not feasible to carry the supplies needed for shelter, devices, containers) Cells originate complex extracellular matrices, complex woods, mineralized structures and many other unique material systems in nature, if we can prepare cells to work, in concert with other preprogrammed cells, so that lyophilized combinations of such cells could be used as on site factories, for *in situ* production to generate complex structures (for example, to generate tissue, a device, a house, a post, etc.).

Challenge: Speeding up Cell Functions as Factories – In many cases, cells are the machines driving advanced biomanufacturing, either directly or indirectly. Cells, depending on the type, function at specific metabolic rates and are generally limited by fundamental mass transfer constraints. Can cells be redesigned, or synthetically designed, to overcome some of the current limitations, in order to improve the kinetics of production of building blocks or other products.

Challenge: Programmed Building Blocks – The ability to ‘build-and-go’ by designing biological legos that have all of the encoded information needed for self-assembly into complex patterns and forms would jump start many applications in advanced biomanufacturing. This is a hallmark of biological materials and emulating and harnessing these features would enhance the formation of complex materials.

Challenge: Biomolecular precision for hard materials – The goal is to combine the spatially precise organization power of biomolecular self-assembly with diverse functionality of hard materials, including metals, semiconductors, etc. Despite many challenges, recent progresses of organizing inorganic materials using biomolecules pictured the exciting possibility of large-scale biomolecule-directed self-assembly of functional devices (e.g. in nanoelectronics, photonics, plasmonic and photovoltaics) with unprecedented precision, throughput and at low cost in future. We need to investigate more effective approaches for interfacing biomolecules with hard materials.

Challenges - Modeling and Simulation

Challenge: Modeling and Simulation – The ability to predict design-assembly rules, hierarchical assembly, scaling of processes and general predictive tools remains primordial. The topic of modeling and simulation is ripe for a robust initiative applicable to every aspect of the field of advanced biomanufacturing, as outlined in the four sessions. Specific challenges could be embedded in each of the four subthemes (e.g., develop a predictive tool to determine how a polymer sequence will self-assemble into a macroscopic material), or these initiatives could be more global in nature (e.g., develop an algorithm that can input primary structure or sequence and predetermine two orders of magnitude in scaled assembly what structure will look like; or – develop a predictive tool that will guide the design of a fundamental biological building block to form a porous structure with specific performance such as mechanical compression; etc.).

Challenges - Education and Training

Challenge – Education and Training – New modes to educate the next generation of students and employees at all levels are needed, where interdisciplinary themes originate from biology and migrate through engineering. This includes developing a common language for the field so that communication among disciplines is meaningful and synergistic. In the same direction, there is a need for new frameworks for transferring knowledge from modeling and simulation to manufacturing of functional biological assemblies.

Challenge – Managing Intellectual Property in Foundries – With core foundries and related themes emerging as important in the field, new strategies to manage intellectual property become critical.

Current Corporate Activities in Advanced Biomanufacturing in the United States

We confined our listing to the US due to practical limitations in tracking the current state of the art. We also followed a similar outline to the four scientific and technology sessions to marry the industrial state of the art to the current/future thinking presented later in this report. Also, this listing is not complete and only identifies some of the active corporate activities to indicate where things stand in the US.

Company Name	Contact Information	Company Description (from web sites)
Cells		
Genomatica	info@genomatica.com (858) 824 1771 www.genomatica.com 10520 Wateridge Circle San Diego, CA 92121	Genomatica delivers new manufacturing processes that enable its partners to produce the world's most widely-used chemicals from renewable feedstocks, with better economics and greater sustainability than petroleum-based processes.
Ginkgo BioWorks	info@ginkgobioworks.com (877) 422 5362 www.ginkgobioworks.com 27 Drydock Avenue, Fl 8 Boston, MA 02210	Ginkgo engineers utilize computer aided engineering and methodologies to produce organisms designed to specification using a growing collection of re-usable genetic parts and host strains to meet customer needs.
Polymers		
KeraNetics	(336) 725 0621 http://dnn6.keranetics.com Richard Dean Research Bldg, Ste 168 391 Technology Way Winston-Salem, NC 27101	Keranetics is an advanced biomaterials company focused on creating innovative keratin-based products for therapeutic and regenerative medicine.
Refactored Materials	info@refactored.com 409 Illinois Street San Francisco, CA 94158	Refactored Materials takes high value natural materials that are too scarce or expensive to extract from nature and uses a combination of synthetic biology and microfabrication to create commercially scalable products.
Tissues		
Organovo	www.organovo.com 6275 Nancy Ridge Drive, Suite 110 San Diego, CA 92121	Organovo designs and creates functional human tissues using our proprietary three-dimensional bioprinting technology with the goal to build living human tissues that are proven to function like native tissues.

Modern Meadow	info@modernmeadow.com www.modernmeadow.com 1601 S. Providence Rd. Columbia, MO 65211	Modern Meadow applies the latest advances in tissue engineering to culture leather and meat without requiring the raising, slaughtering and transporting animals.
Tengion	(336) 722 5855 www.tengion.com 3929 Westpoint Blvd, #G Winston-Salem, NC 27103	Tengion is a clinical stage regenerative medicine company discovering and developing regenerative products. Tengion is pioneering the development of products comprised of a patient's own regenerative cells, with or without a biocompatible material component that are implanted into the body.
Organogenesis	(781) 575 0775 www.organogenesis.com 150 Dan Road Canton, MA 02021	Organogenesis is a leading regenerative medicine company with the unique skill set to take complex living therapies from research and development through manufacturing to successful commercialization.
Cytograft	contact@cytograft.com (415) 506 0260 www.cytograft.com 3 Hamilton Landing, Suite 220 Novato, CA 94949	Cytograft is an established leader in the field of cardiovascular regenerative medicine. Using cells harvested from the patient or another human donor, we are able to repair diseased cardiovascular tissues and organs.
Humacyte	(919) 313 9633 www.humacyte.com Morrisville, NC 27560	Humacyte develops novel human tissue-based investigational products that are being developed for potential commercialization for key applications in regenerative medicine and vascular surgery.

AGENDA - NSF Workshop [July 25, 26, 2013]: Advanced Biomanufacturing

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DAY 1 - July 25 (Thursday)**1:00 PM - Workshop Opening**

- Welcome, Initial Guidance
- Remarks from NSF Acting Division Director of Division of Chemical, Bioengineering, Environmental, Transport System (Robert Wellek)
- Short talk from the FDA (Mark Lee)

2:00-5:00 PM - Opening Sessions

- Co-chairs for each Session (4 groups) - present pre-organized overviews – emphasizing state-of-the-art, barriers, key questions to address gaps in knowledge, advances needed to move forward as part of a roadmap, cost and regulatory issues
- Questions/discussion/inputs from the larger workshop attendees
- Outcome → Each team will provide topics and recommendations to the larger workshop, as well as their initial roadmap of issues to frame discussions

3:00-3:15 PM - Break**5:00-5:30 PM – Brief Remarks**

- Pramod Khargonekar, NSF Assistant Director, Directorate of Engineering
- Federal Program Directors – short overviews of programs, plans, interests

5:30-8:00 PM - Working Dinner

- Workshop participants to discuss the topics from the afternoon, gather input, plan for Friday
- Stephen Lehrman, Legislative Assistant, Office of Senator Mark Pryor

DAY 2 – July 26 (Friday)**8:00 AM – Breakfast****9:00-12:00 AM - Breakout Sessions**

- Co-chairs organize their teams to refine inputs, update documents based on the discussions from Thursday
- Co-chairs organize initial write ups – power points and text

10:00-10:15 AM - Break**12:00-1:00 PM – Lunch****1:00-3:00 PM - Large Workshop Group Meeting**

- Co-chairs present findings for additional feedback, lead discussions, identify interfaces with the other subgroups

3:00-3:15 PM – Break**3:00-5:00 PM - Breakout Sessions**

- Finalize plans from each team

- Outcome → cochair and teams draft team inputs

WORKSHOP TOPICS, SESSIONS, CO-CHAIRS

Organizing Committee – Co-Chairs:

- Gang Bao (Georgia Tech) (gang.bao@bme.gatech.edu)
- Chris Bettinger (Carnegie Mellon) (cbetting@andrew.cmu.edu)
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Conference Program: [* = cochair]

Molecular Approaches and Building Blocks

Overview - At the molecular level, rapidly advancing tools have empowered the ability to design and implement new cell capabilities, including cellular re-wiring, the introduction of new metabolic pathways, the synthesis of biological building blocks or components for more complex materials, and expanding the toolkit from nature to synthetic options such as nonnative amino acids and modified sugars. These features permit the generation of useful components and pathways towards new monomers for building polymers or for functionalizing devices or systems. *Examples:* Reprogramming Cells, Synthetic Biology, Metabolic Engineering, Biological Building Blocks, Nonnative Amino Acids, Nonnative Saccharides, Inputs to Polymeric Materials

Participants and Cochairs:

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Cellular Approaches, Assemblies and Polymers

Overview – The exploitation of whole organisms or combinations of organisms to generate polymers. This is an extrapolation of what happens in nature, where genetic and related tools as above provide improvements in polymer yield, chemistry and molecular weight. The ability to couple polymer synthesis to materials formation and function is being pursued in some systems, such as bacterial cellulose and silk-elastin copolymer systems. Additional areas of interest include control of cell patterning and organization related to tissue structure, as processes from developmental biology, including cell-cell interactions, biophysical factors, etc. Bacterial and viral assemblies and functions related to new materials and devices are also under study. Many of these systems rely on self-assembly for cells or virus to organize into patterns with new structures or new functions. *Examples:* Biopolymer synthesis (e.g., bacterial cellulose, polyhydroxyalkanoates, hyaluronic acid, keratin, collagens, silks, etc.), cell patterning and organization, viral assemblies, folding patterns, biophysical factors in patterning

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Tissue/Organ Approaches

Overview – Options to print or assemble tissues and organs have emerged in recent years. This includes various modes of preparing cells/solutions for programmed assembly into arrays in 2D and 3D related to tissue and organ formation and function. *Examples:* 3D printing of cells, tissues and organs

Participants and Cochairs

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Systems Integration

Overview – Systems integration brings together the biological building blocks, polymers, organism assemblies or tissues/organs into functional devices and systems. Identify modes to achieve these goals with full integration. *Examples:* electronic and optical devices, complex systems

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Boland, Thomas	Professor	University of Texas at El Paso
Culler, Stephanie	Research Scientist	Genomatica, Inc
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Densmore, Douglas	Assistant Professor	Boston University
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Forgacs, Gabor	Professor	Clarkson University
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Nerem, Robert	Institute Professor Emeritus	Georgia Institute of Technology
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Session 1 - Molecular Approaches and Building Blocks

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Note: this session was subdivided into 3 areas to reflect the various specialty areas and approaches being pursued:

I. Molecular Building Blocks

II. Challenges of Scale-Up: Moving Strains from Bench to Large-Scale Fermentation and Product Recovery

III. Structural DNA nanotechnology

These three themes reflect where we are now, with respect to:

- Manufacturing applied to organism engineering
- Fermentation to manufacture chemicals/fuels
- DNA-driven manufacturing

I. Molecular Building Blocks: The Role for Synthetic Biology in Advanced Bio-Manufacturing

State of the Art

At the molecular level, rapidly advancing tools have empowered the ability to design and implement new cell capabilities, including cellular re-wiring, the introduction of new metabolic pathways, the synthesis of biological building blocks or components for more complex materials, and expanding the toolkit from nature to synthetic options such as nonnative amino acids and modified sugars. These capabilities permit the generation of useful components and pathways towards new monomers for building polymers or for functionalizing devices or systems.

A substantial fraction of these capabilities are accessed by programming cells with DNA. Cells are provided synthetic DNA that encodes for collections of genes and other genetic elements that work together to accomplish a desired function. To this end, DNA manufacturers already have established production pipelines for relatively small DNA constructs (around 1000 DNA base pairs), and are continually improving their processes. This DNA manufacturing capacity for building polymers or for functionalizing devices or systems (**Figure 1**) has already provided the basis for consumer biomanufactured products in the chemical, fuel, and pharmaceutical industries.

Although there are already commercial applications, the difficulties intrinsic to transitioning complex systems through development stages have hindered molecular biomanufacturing from realizing its full potential (**Figure 2**). Systems containing dozens or hundreds of interrelated DNA elements have the potential to provide a tremendous variety of valuable new functions, chemicals, and materials. However, the development of such systems typically stalls at the proof-of-principle stage due to their complexity. In order to address this barrier, dedicated efforts are necessary to establish a reliable bridge for complex engineered systems to transition from proof-of-principle to production readiness, thereby providing a route for them to enter into existing mainstream biomanufacturing. In order to achieve such a bridge, three distinct kinds of efforts are necessary:

First, there is a continuing need for efforts dedicated to the discovery, characterization, and dissemination of useful DNA sequences (often referred to as genetic “parts.”) The exemplar facility for these kinds of efforts, the BioFab (www.biofab.org), was initiated by NSF in 2009 with the specific mission of creating repositories of DNA sequences that can be easily accessed and re-used for multiple classes of engineering projects. More generally, facilities following this model (referred to here as “**Fabs**”) are needed to build and test large sets of genetic parts emerging from academic research, catalogue their behavior, and centralize their distribution, thereby removing barriers of access to sequences and information.

Second, rapid design and prototyping facilities are needed to determine how to most effectively assemble the parts produced by Fabs into systems that produce desired behaviors. Such facilities, referred to here as “**Foundries**,” address the intrinsic contextual complexity of large biological systems via high-throughput design-build-test-learn cycles. To do this, Foundries design and build large sets of combinations of genetic parts, test all the combinations, and then apply learning algorithms to extract assembly rules to enhance function. By doing so, Foundries can leverage knowledge from Fabs to shepherd complex systems from the proof-of-concept stage to one suitable for production. Moreover, Foundries will provide a critical role in technology dissemination of techniques for design and prototyping by industrializing early-stage DNA-manipulation techniques from academia and providing associated training to scientists in manufacturing industries, and by generating demand for such techniques in manufacturing settings by establishing manufacturing viability for complex, previously inaccessible systems. Recently, DARPA has announced its intent to fund Foundry-like efforts focused on the synthesis of small molecules; this initiative will start in the latter half of 2014.

Third, the exchange of information between Fabs, Foundries, and manufacturers that utilize their output will rely critically on **Metrology**. Metrology refers to the development of standards for the measurement of the behavior of biological components (including “parts”), the descriptions of components (such as sequence, necessary context, meta-data etc.), ontologies, methods, models, quality metrics, and software specifications, such as for data interchange. At the time of this writing, little formal metrology work has been funded in this area, although NIST/ABMS have recently sponsored a workshop (July 2013) to establish a roadmap for metrology in synthetic biology.

Although they will fulfill a critical niche to enable molecular biomanufacturing, the establishment of Fabs, Foundries, and Metrology are all at nascent stages. It is important to note that initiatives such as these generally fall outside of the conventions of traditional academic research or industrial R&D. However, there is strong precedent for investment in such infrastructure: The establishment of factory-scale academic DNA sequencing centers has revolutionized biomedical and pharmaceutical R&D. Similarly, continued investment in transitional infrastructure will enable more complex, next-generation molecular approaches to fully leverage already-established biomanufacturing infrastructure.

Prototype & Process Manufacturing

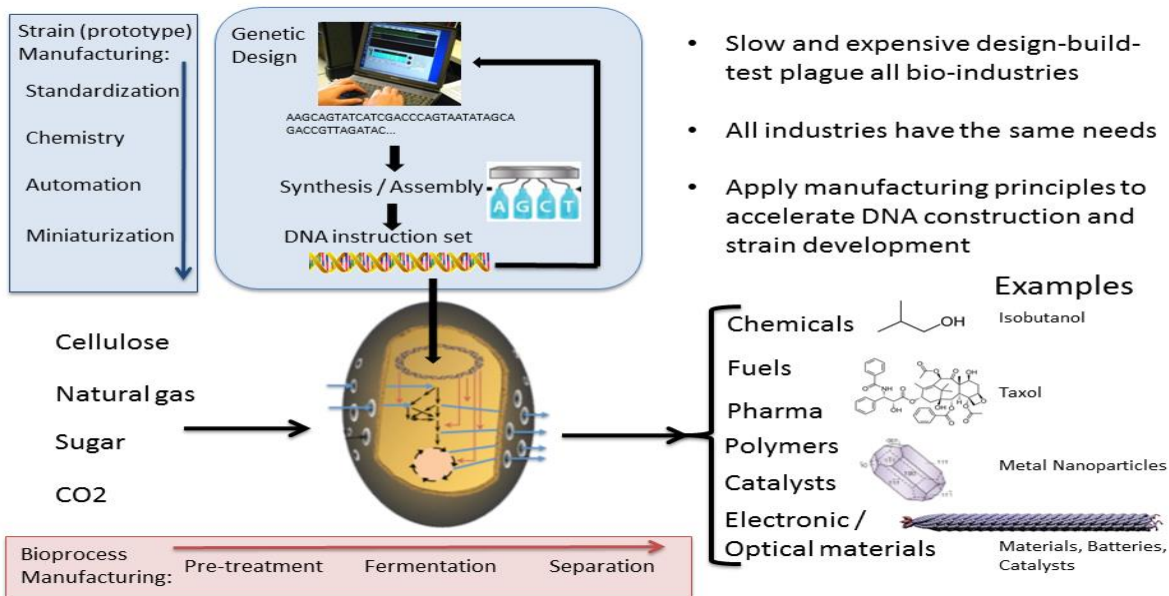
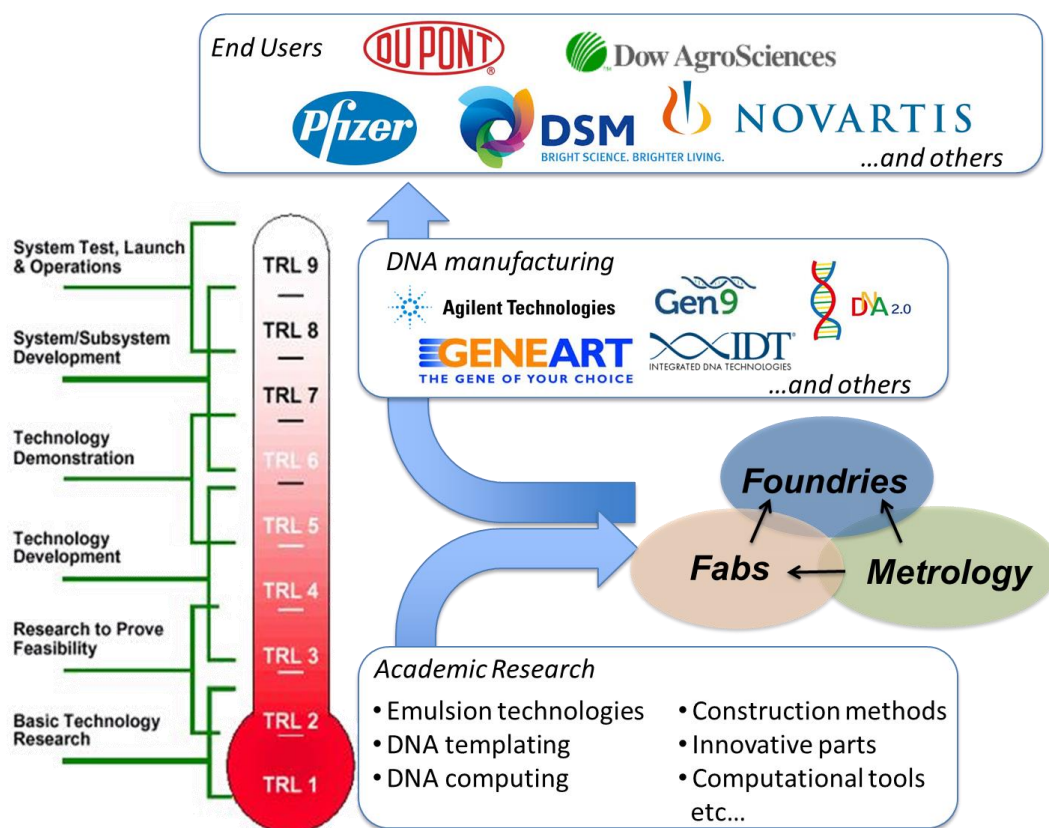


Figure 1. Aspects of biomanufacturing processes. Although biomanufacturing applications are diverse, including chemicals, materials, fuels, pharmaceuticals and other areas, they all utilize very similar processes. Genetic designs are synthesized into physical DNA molecules, which are then incorporated into cells. The cells are then provided with relevant feedstocks and nutrients and cultured in an environment that maximizes their productivity. Desired products are then separated and purified, and then processed for their intended application.

Figure 2. Illustration of proposed flow of molecular biomanufacturing technologies necessary to facilitate transition from proof-of-principle through DNA manufacturing to end-users in industry. There is a critical infrastructure need for “Fabs” to populate the space of biological parts, for “Foundries” to design these parts into large systems, and “Metrology” to establish common measurements and standards for the exchange and dissemination of parts and designs.



Summary of organism level state of the art:

Organism Engineering

- Genes
 - Individual gene design (codon optimization for expression)
 - Mutant libraries diversifying amino acid composition of proteins (protein engineering / machine learning)
 - Active manufacturing pipelines at companies for small to mid-size oligos (up to 500 bp) to single genes (Agilent, Geneart, Gen9, Genscript)
 - Chip-based oligo synthesis up to 200bp (250,000); Gene cost drop (3X)
- Pathways
 - Larger DNA assembly technologies based on restriction enzymes and manual molecular biology
 - DNA constructs built using oligo-bridging, Type IIS restriction enzymes (Golden Gate), exonuclease-based methods (Gibson)

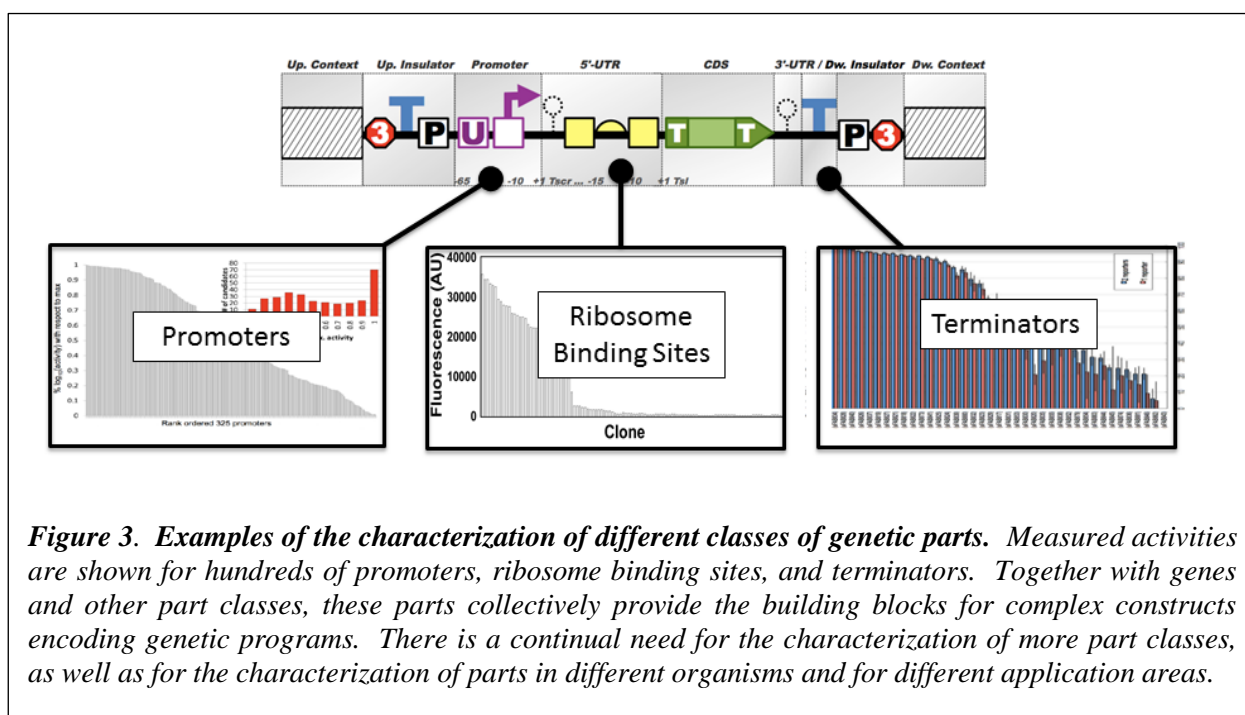
- Sequence verification via multiplex PCR, sanger sequencing, and deep sequencing
- Automation by liquid handling systems
- Low-throughput and species-specific DNA transformation.
- Genomes – Mycoplasma (whole genome synthesis), Synthetic Yeast Genome (Piecemeal replacement), re. *E. coli* (Piecemeal replacement)

Gaps

- Whole genomes can be built but not predictably designed
- Many sophisticated multiplexed genetic systems can be designed but not built
- Creation of meaningful genetic diversity for large designs containing a massive number of parts
- Depth of sequence databases and a capability to access this as a resource (homology only)
- Large set of target organisms with arbitrary design and scale-up needs. Non-transferrable data and rules.
- Co-navigation of genetic space with environmental/strain variability

Barriers

- Transformation methods that are applicable to broad species diversities
- Access to strain resources. Nationalized resources are highly restricted. Small companies are unable to get access to strains without inhibitory licenses. The field would benefit from a national strain bank from public lands that has a low barrier to access for exploitation.
- Lack of standards:
 - Part characterization (**Figure 3**)
 - Verification and quality control and process standards (e.g., communication to robotics or metrology for the process itself).
 - Data set integration: transcriptomics, proteomics, sequencing, verification, and screening
 - Software communication between Computer-aided design software, Laboratory Information Management Systems, and Issue/Project Tracking Systems.
- Challenge of having to screen large number of constructs in the wrong conditions (microtiter plates versus large fermenters).



Art of the possible

- Organism Foundries: Move laboratory processes to from R&D to manufacturing footing and then SCALE
 - Done before with genomics
- High-throughput capacity to mine and screen biocatalysts
 - Monetization of sequence information resources
 - Characterize the ‘natural part set’
- Standardize the chassis organism. Lock in the fermentation and organism and set the design and operational rules for this organism. How do we know when an organism is sufficiently characterized to be a “platform.”

Modeling and Simulation

- No effective means of linking sophisticated cellular models (metabolism, synthetic biology) with models to guide scale-up
- Difficulty in acquiring data to inform the process of scale-up

Training and Education

- Education of biotech workforce at large companies in the potential of modern genetic design/synthetic biology
- Teaching automation/process/manufacturing with backgrounds biology and vise versa.

- Technicians to operate organism engineering foundries

II. Challenges of Scale-Up: Moving Strains from Bench to Large-Scale Fermentation and Product Recovery

Advanced biomanufacturing of chemicals and materials requires the development of robust approaches for the design and construction of microbial strains with validated performance at scales ranging from 1 ml to 100,000 liters (9 orders of magnitude).

The state of the art

Strain and process development cycles are linked by the sensitivity of strain performance to changes in process conditions. As a result, the transfer of laboratory validated strains to commercial scale is a major challenge in biomanufacturing today. While there are standard metrics that are known to affect performance (such as oxygen transfer rates), the complete collection of environmental variables along with how they affect metabolism remain poorly understood. Additionally, the identity and relationships among the genes, pathways, and complexes related to such metabolic alterations are not well characterized. The outcome is that scale-up is often more art than science, is low-throughput, expensive, and time-consuming.

The state of the art in scale-up has not changed considerably over the past few decades even through biotechnology capabilities have advanced by orders of magnitude. The typical scale-up operation involves i) rounds of 96-, 28-, and 24-well plate based assays on targeted strains under various conditions followed by ii) rounds of 1-10L fermenter studies with greater control and monitoring. This process is sometimes performed recursively with strain design and at various scales (**Figure 4**).

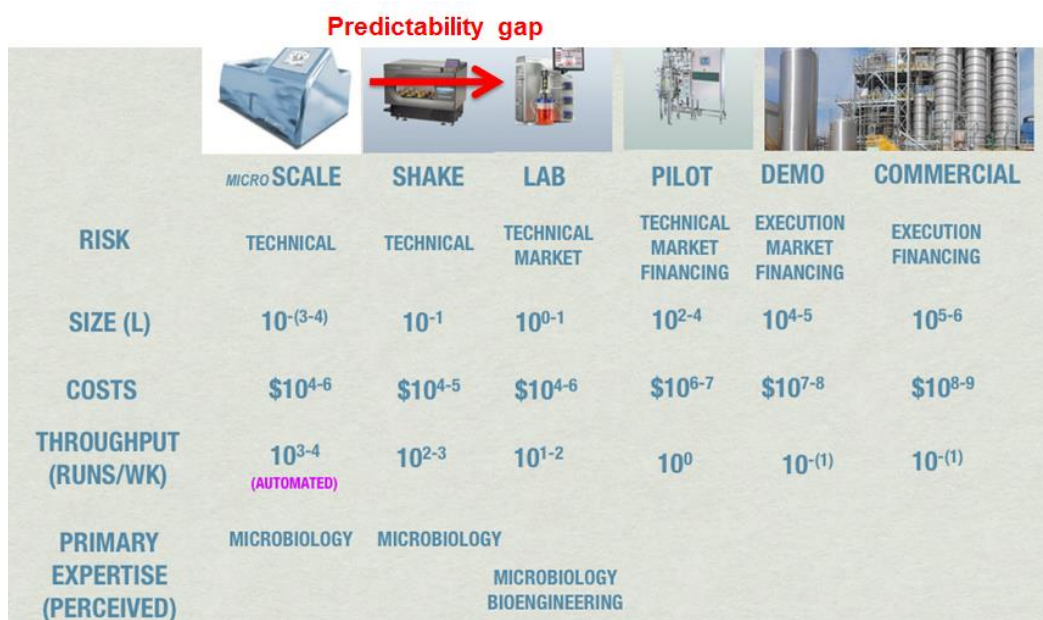


Figure 4. To make fermentation scale-up reliable the growth and product production performance of strains at smaller scales must be indicative of performance at larger scales. Of the 6 scales of fermentation shown above there was a clear consensus that the largest gap in predictability is between microscale (10^{-3} L) and lab (10 L) scale. Shake flask scale (1 L) is ineffective and should be removed. New research is needed to improve the quality of microscale fermentation so that strain performance at microscale translates directly to performance at lab scale.

Gaps and barriers

- Mapping of < 1 ml to 1-10 L cultures. We currently have no standardized approaches for mapping the performance obtained at microscale to the 1-10 L scale (mapping from 1-10 L to 10,000-100,000L scales is much more accurate). This necessitates the performance of a large number of fermentations run at all scales, and because of differences in throughput this slows the development process considerably.
- Microscale studies are limited to ~1 ml and 1000-10,000 samples per run. 1-10L studies are limited to 100-200 runs per week. Given massive improvements on the horizon for genome design and construction (~10,000 designs / month), and the need to test many different conditions (~100 or more), scale-up / fermentation throughput is well below what is required.
- Assay throughput and accuracy: cannot measure range of things we would like to measure at smallest scales. Throughput of offline measurements then become rate limiting.
- Understanding of microscale to mesoscale environmental differences and what differences control strain performance. Need better methods for studying this issue.
- number of fermenters that can be run by single individual is too low and not well enough automated. Moreover, the cost of individual fermenters and fermentations remains high.
- Lack of a standard, validated, user friendly economic models that are tied directly to genome designs and validated performance of those designs
- Sensors and screens for individual molecules are missing.

- Better understanding of existing and needed larger scale fermentation facilities is needed. Additionally, need improved understanding of how to retrofit existing facilities (e.g., ethanol).
- The extent to which private industry would support a government facility that massively reduces early stage scale-up risk and open-sources the data is not clear (removal of intellectual property and/or business models involving these stages).

Art of the Possible

- Major effort for identifying environmental and genetic factors that enable micron scale to 1-10L scale mapping of strain performance. This effort would involve a range of technologies; building off of advances in synthetic biology, high-throughput assays, and systems biology.
- A capability to perform 1000's of 1-10L fermenters in a week. This capability would likely take advantage of the latest laboratory automation and systems monitoring technologies, with the goal of increasing throughput and reducing management needs by orders of magnitude. [The fermentor capabilities should be adaptable enough to cover a larger fraction of process design space (airlift vs aerobic vs anaerobic) and design of experiments should play a role in utilizing this capability]
- The facility should allow for future collaborators/users to pick, plug, and play validated genome-designs (i.e. open sourcing of platform strains, or designs, producing various intermediates (acetyl-CoA, pyruvate, etc.)
- The facility should be a leader in developing protocols and standards in measurements of performance at all scales.
- The facility should produce validated, standardized, and sophisticated technoeconomic models that are used early on to define process space, which then shapes designs, and design of experiments.
- The facility should consider how to handle multiple different feedstocks (e.g., sugar, cellulosic, gas)

Role of modeling and simulation

- This is viewed as an important tool that should be integrated within the facility analytical toolbox.

Regulatory and cost issues

- The changing importance of renewable chemicals in a climate change policy environment (i.e mandates in Europe or other regions) was noted.

Training and Education

- The facility should develop programs that aid in the training of the next-generation of “metabolic engineers” that have understanding ranging from genome design and construction through metabolism and bioprocess engineering. The programs should be creative, extend well beyond the boundaries of the facility (international), collaborative, and make use of modern teaching technologies (web based massive online courses, tutorials).

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III. Structural DNA nanotechnology

State of the Art

Digital fabrication, in comparison to analog fabrication, is more powerful and versatile due to its modularity and high accuracy (**Figure 5**). Nucleic acids, especially DNA, have been used by nature as digital molecules for programming cellular behaviors in biological systems. Not focusing much on DNA's biological properties, structural DNA nanotechnology instead tries to harness the power of digital self-assembly and fabrication. Founded three decades ago, the field has grown rapidly and become an effective approach constructing sophisticated synthetic molecular structures and devices.

Researchers have created diverse synthetic nucleic acid structures such as lattices, ribbons, tubes, finite 2D and 3D objects with defined shapes, and macroscopic crystals. Many dynamic devices have been constructed in parallel, including tweezers, switches, walkers, and circuits. Last year the field made a few breakthroughs in digital self-assembly of nanoscale 2D, 3D, and microscale crystals using "DNA bricks" as building blocks. In all of these cases, the resolution approaches 2-nanometers. Additionally, as DNA and RNA can be interfaced with other functional molecules in a technologically relevant fashion, synthetic nucleic acid structures promise diverse applications; researchers are using DNA/RNA structures and devices to direct functional material arrangements, to facilitate Nuclear Magnetic Resonance (NMR) protein structure determination, to develop bioimaging probes, and to organize and regulate molecular pathways in living cells.

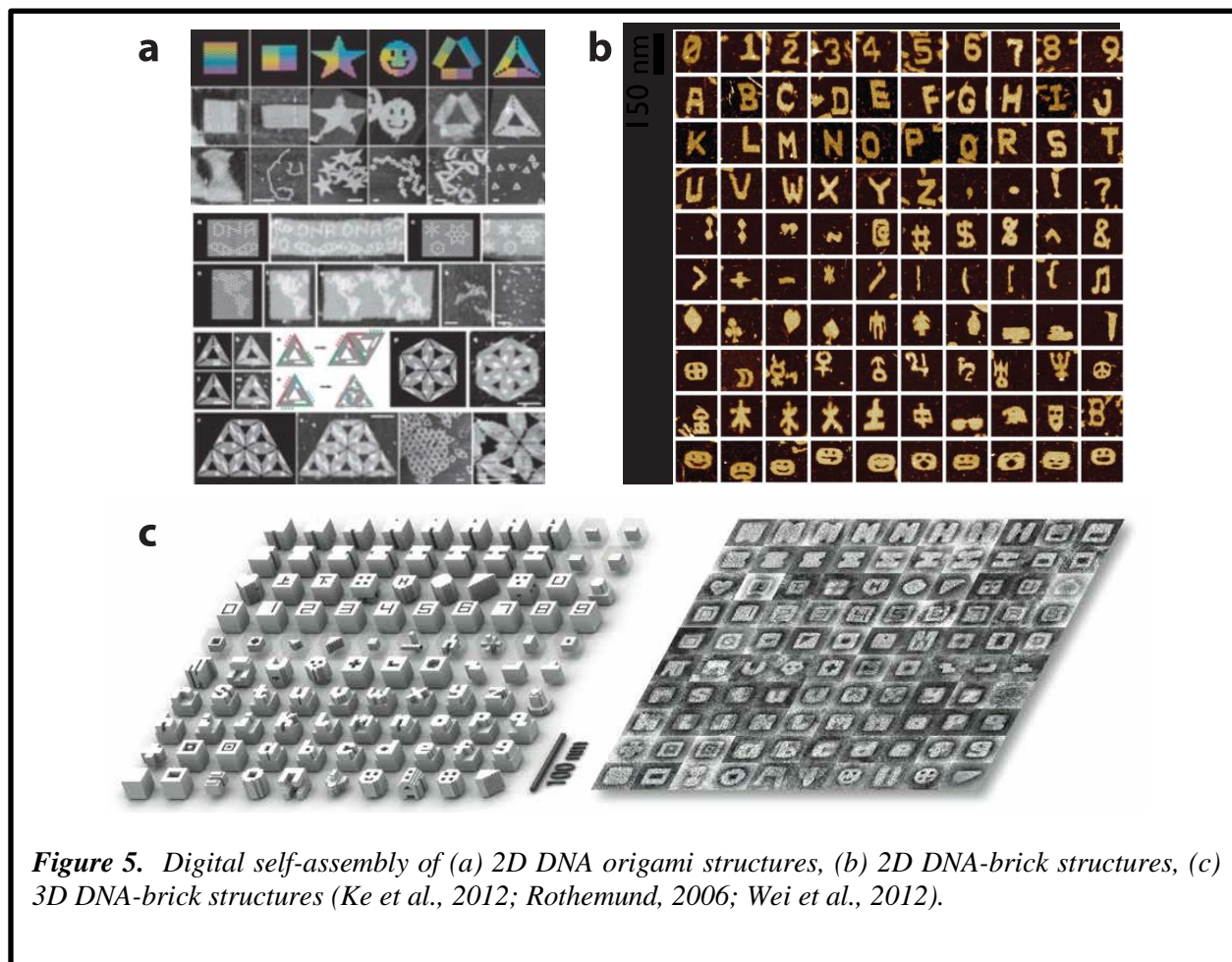


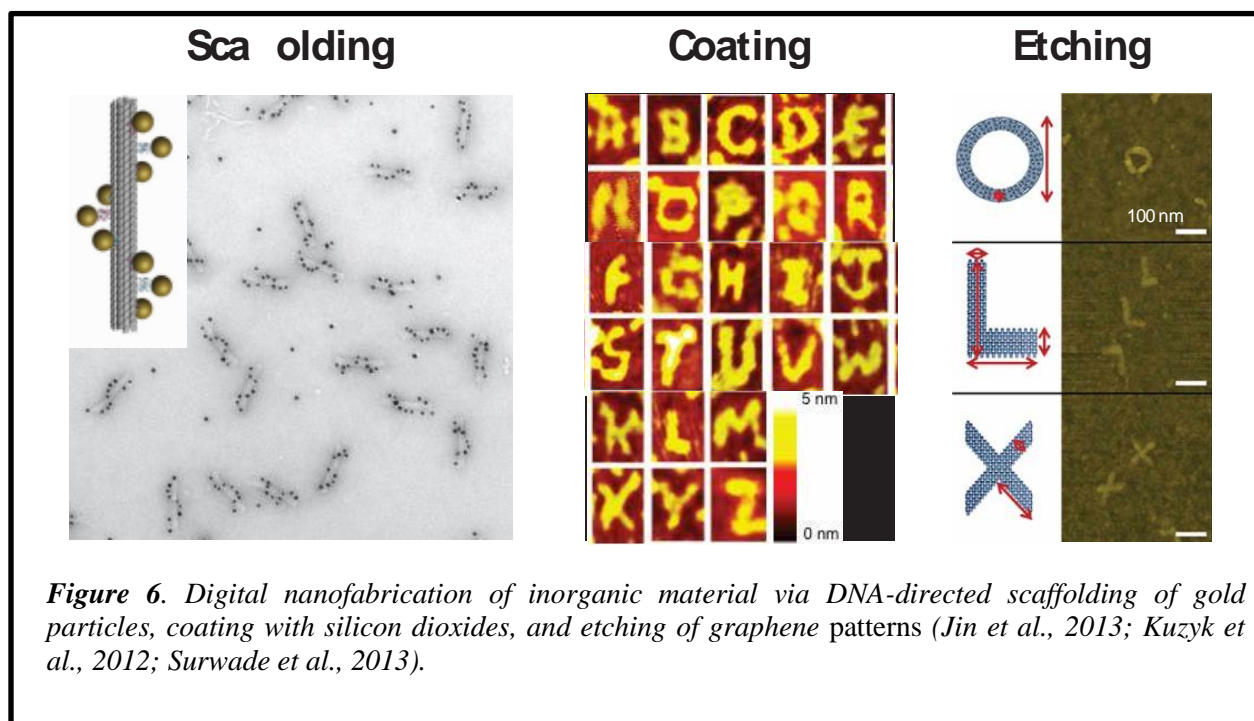
Figure 5. Digital self-assembly of (a) 2D DNA origami structures, (b) 2D DNA-brick structures, (c) 3D DNA-brick structures (Ke et al., 2012; Rothmund, 2006; Wei et al., 2012).

Digital fabrication of inorganic materials (Figure 6) - Could we combine the power of digital self-assembly with functionality of many other functional materials that are widely used in industry, especially inorganic materials, such as semiconductors, metals, and carbon-based materials? In a way, this is a similar challenge that the top-down 3D-printing technology has to address: how to rapidly prototype a wide range of materials to achieve desired functions. Recently, a few publications have demonstrated pioneering work of transferring structural information of digital DNA self-assembly to functional materials, including metallic nanoparticles, metal oxides, graphene, through a variety of processes. The typical resolution of the final structures is around 10-20 nanometers.

The semiconductor industry and other emerging applications, such as nanophotonics and nanoelectronics, are in constant pursuit of low-cost, high throughput manufacturing of materials/devices at smaller and smaller scale. Digital DNA self-assembly offers an alternative, promising route to conventional top-down lithography. First, it can potentially assemble materials at sub-5 nanometer resolution. Second, self-assembly is a parallel process. Millions or even billions copies of same shaped products can be produced. Third, 3D materials/devices can be assembled in a single-step, unlike the conventional lithography 3D manufacturing, which typically requires multi-step, layer-by-layer processes.

The fruits of DNA-directed digital fabrication are not yet mature enough for large-scale manufacturing. What needs to be done in order to capitalize on its potential? Three imminent challenges have to be overcome:

- (1) develop either chemical or enzymatic methods for high-quality, low-cost, large-scale production of DNA or RNA
- (2) reduce the loss of resolution during the fabrication down to a few nanometer or even angstroms
- (3) interface with a wider range of materials and develop multi-component fabrication approaches - molecules and materials that can currently be controlled include proteins, nucleic acids, some small molecules, nanoparticles, graphene, semiconductors.
- (4) improve device performance by using highly crystalline precursor or post-treatment to improve crystallinity under extreme conditions.



Gaps and Barriers

- Self-assembled DNA structures with high complexity, resolution, and precision.
- Transfer of the spatial information to more diverse technologically relevant functional materials with high accuracy and resolution.
- Move from simple prototype (e.g. etching a simple graphene ribbon and producing a single field effect transistor) to integrated functional structures and devices (e.g. etching wafer size integrated circuits); developing other “killer apps”.
- Low cost, high quality, long sequence DNA and RNA production at large scale
- Effective computational tools for designing and analyzing the self-assembled structures.
- High resolution, accuracy transfer of the spatial information to technologically relevant functional materials.
- Stability of self-assembled DNA structures under extreme conditions.

Art of the possible

- Discrete, uniquely addressable structures over 1 micron size; extended crystals with repeating structural units that grow to over 100 micron size with complex geometrical features; millimeter to centimeter surface area with 5 to 10 nm uniquely addressable features
- Transfer the spatial features of synthetic biomolecular structures to diverse technologically relevant materials with complex features with nanometer resolution and over micron to millimeter area.
- Tunable thickness and composition of DNA crystals to enhance the resistance at extreme conditions.
- High value applications for the semiconductor industry (e.g. wafer size lithography masks with sub 5-nanometer complex features), electronics (e.g. graphene based electronic circuits over large area), photonics (e.g. metamaterials with precisely tunable optical features, such as negative index, in the visible wavelength), spatially organized protein nanofactories for production of useful products in vitro and in vivo.
- Programmable molecular instruments for molecular diagnostics and therapeutics

Modeling and simulation

- Existing design & analysis tools: CAD design tools with user friendly interface, software tools for liquid handling robots, sequence design tools based symmetry minimization or thermodynamics; Programs that use experimentally attained information and simple elastic model of DNA duplex to simulate twisting, bending. Computational prediction of deformed DNA shapes
- Challenge: More sophisticated and powerful design and analysis software tools that have much-needed functions, such as rapidly simulation lowest-energy state of large structures; designing complex dynamic self-assembly of structures.
- Challenge: Computer tools that fully automate the integrated design, construction, and test cycles.
- Challenge: Design and analysis tools for materials beyond DNA structures, e.g. for DNA-templated inorganic structures and devices.

Training and Education

- Interdisciplinary graduate training is crucial for the next stage development of the nucleic acid nanotechnology. Integrated efforts between DNA nanotechnologists, physicists, chemists, biologists, and engineers will be essential for realizing the full potential of DNA-directed biomanufacturing.

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State of the Art

The ability to use cells, organisms or communities of organisms to generate polymers and structured, active materials is one of the hallmarks of biological systems. In particular, cells can generate polymeric materials with diverse properties for biomanufacturing devices or cell-material assemblies (**Figure 7**). These biologically derived materials have a number of advantages that include enhancements in chemical uniformity, defined molecular weight and monodispersity, and controllable physicochemical properties. Further, they provide scaffolding in which cellular assemblies can develop to create organized tissues, structured chemical factories, and organized structures for biomedical applications and beyond, such as energy storage and generation.

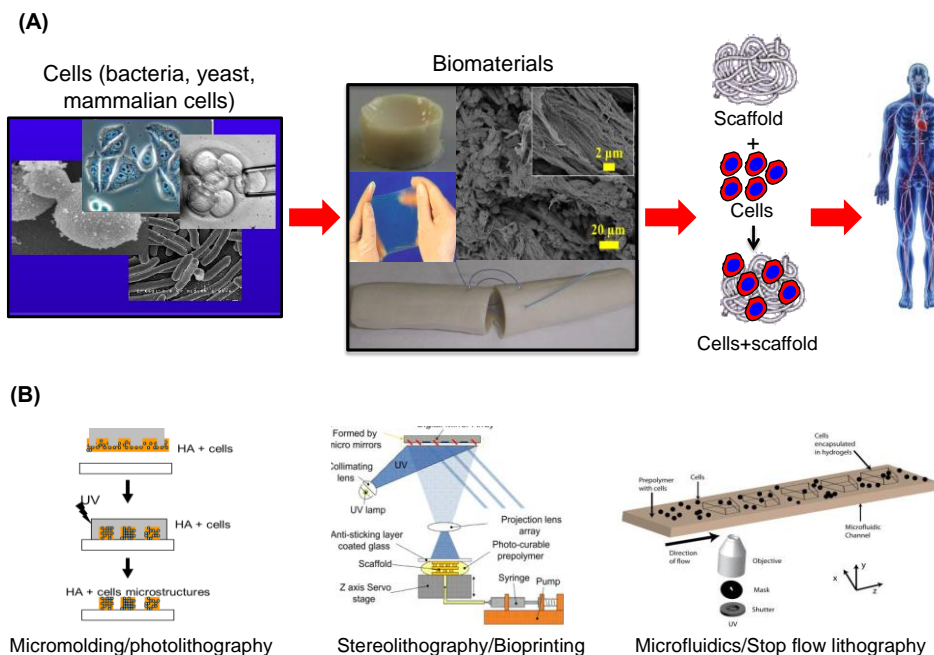
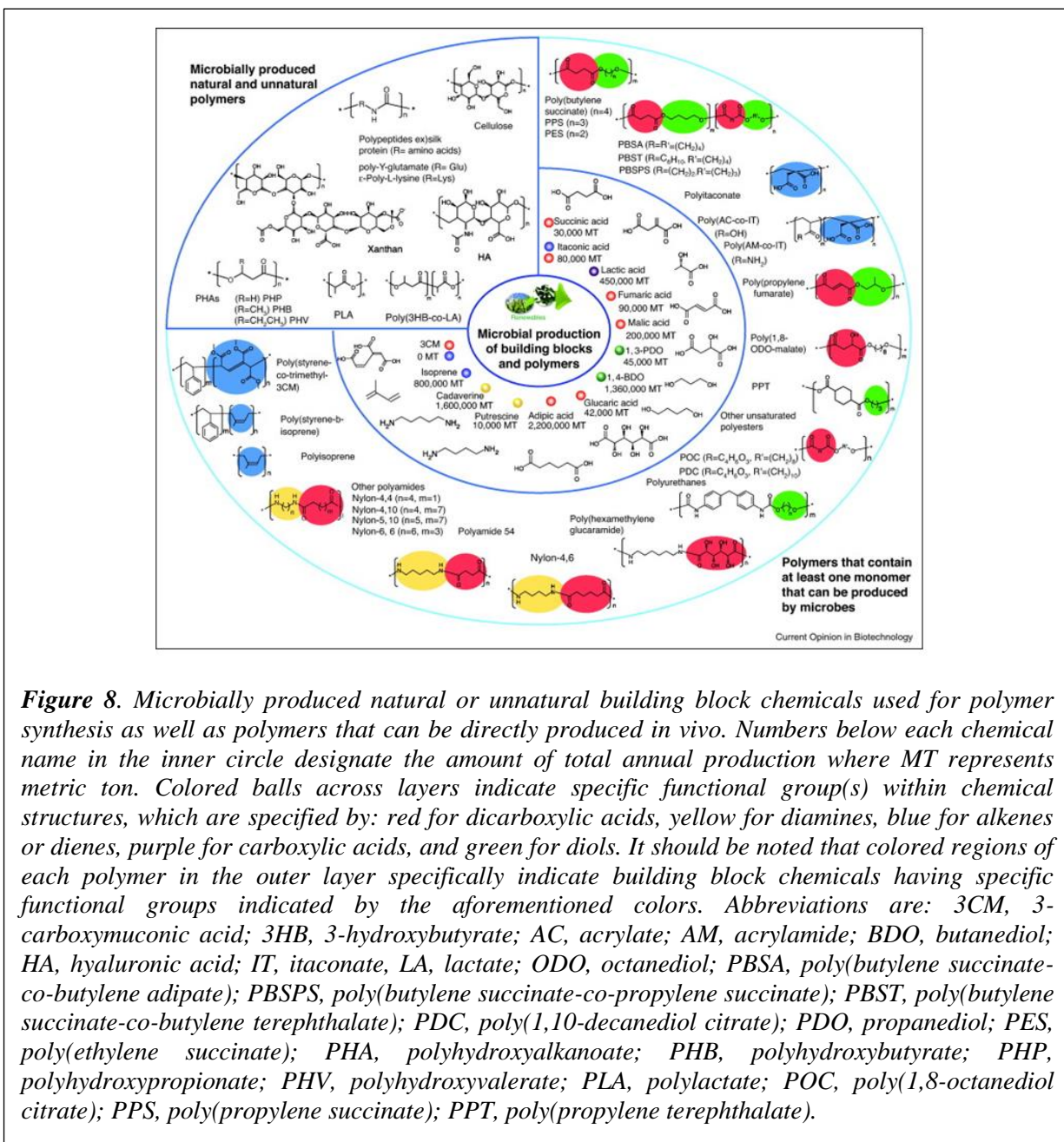


Figure 7. (a) Organisms to generate biomaterials for tissue regeneration, (b) microscale technologies to control cell organization and cell-cell interactions.

Extant biomanufactured materials are already successful. For example, elastin-based materials provide incredible resilience and elasticity to biological tissues. Silk-based materials have incredible strength. Other types of natural materials such as polysaccharides and collagen also provide unique and important properties. These types of materials can be derived either from biological sources or can be made using recombinant techniques (**Figure 8**). When cells secrete or interact with these biomaterials, they have been coaxed to form functional human and animal tissues, structured microbial biofilms that are protected against material degradation or form self-healing materials, or highly organized viral assemblies that can act as flexible piezoelectric materials.



The design and manufacturing processes for the assemblies of these biomaterial and active cellular and viral components are currently heterogeneous, primitive relative to other manufacturing disciplines, and not standardized. The processes span molecular design, biological pathway design, materials design, and design for overall assembly of the complex mixture of cells and materials. There is an opportunity to integrate theory, computation, characterization and physical manufacture to improve the scalability and reliability of the biomanufacturing process.

Gaps

- Interrelationships.** The interrelationships among the biomechanical/biochemical/bioelectrical factors, cell/tissue microenvironment, biology/physiology, and implant/tissue integration must be better elucidated. It is far from clear how microenvironmental cues (such as, soluble chemical factors, ions, local stiffness, microarchitecture, topography, porosity, diffusivity, and their gradients) influence cell machinery to produce proteins and polymers, or to remodel the environment resulting in new biomaterials. As the cells enter a given microenvironment, their interaction with that environment leads to self-assembly, organization, patterning, and cell-cell signaling. Despite significant advances in biology, much of the underlying principles are not known. Understanding will also require significant effort directed at understanding the ion-flux dependence of relevant processes such as secretion, proliferation, differentiation, migration, apoptosis, etc. These gaps in our knowledge limit our ability to fully exploit these processes and to develop the predictive models that are essential for the manufacturing of these polymers. Once these dependencies have been better described, the door will be open to important advances such as live-cell mediated delivery systems capable of targeting specific sites with reagents for both enhancing (e.g., inducing tissue generation, etc.) or inhibiting (e.g., drugs for cancer, cardiovascular disease, diabetes – related diseases, etc.), as well as improved implant biocompatibility and tissue integration. Cell generated polymeric biomaterials may be passive, or they can serve as live, active, hybrid bio-polymer with specific functionalities. These hybrids may self generate, self heal, and continue to emerge and evolve refined functionalities, depending on their usage.
- Moving from lab protocols to assembly lines.** The success of various types of biomanufacturing processes is still out of reach partly because some fundamental understanding of the key elements of these processes is still missing. For example, in tissue engineering, scaffold degradation *in vivo* is often predicted from the outcomes of *in vitro* degradation studies, and these are often not correct. However, direct quantitative determination of degradation *in vivo* has been problematic due to the difficulty of separating the infiltrated/regenerated tissues from the porous scaffolds, thus the predictions remain untested and still require *in vivo* quantitative validation. It is imperative that we find *in-situ* real-time methods to facilitate tracking or monitoring the dynamic changing tissue regeneration and scaffold degradation processes without sacrificing animals. This issue has rarely been addressed, thus, the field of tissue engineering remains a trial and error process, to some degree. New biomaterial tools, engineering methods, design principles, non-invasive, and real-time assays are urgently needed to move the field of tissue engineering forward.

Ex-vivo storage/preservation technologies are lacking for off-the-shelf tissue grafts that incorporate live cells. Measuring tools and methods of quality assurance for biomanufactured tissue grafts in storage must also be developed before the biomanufacturing process can be used to develop tissues suitable for in-situ deposition.

- **Functional heterogeneous multiscale biomolecule or tissue assemblies.** Manufacturing of heterogeneous and multiscale structures that achieve desired compositions, architecture, functionality, and chemical and physical properties is not currently possible because of a lack of study into how different manufactured assemblies interact when combined and how structural stability and viability are maintained. The use of these mixed biomaterials will be necessary to support the full spectrum of cell types and behaviors required to meet the promise of advanced biomanufacturing.

Barriers

Lack of sufficient theoretical and computational modeling and scaling up for advanced biomanufacturing technologies. Computer aided theoretical and computational models, computer-aided design of integrated systems, and modeling of biopolymer materials and cellular assemblies will play a critical role in generating testable hypotheses based on realistic principles. However, neither the computational nor the mathematical theory currently exists in sufficient detail to actively contribute to the experimental process. Multiscaling, multiphysical and mixed-abstraction modeling, with uncertainty and big data management, remain a challenge.

Recommendation: Encourage interdisciplinary studies comprising mathematical modelers, biologists, and bioengineers; including experts in other fields, such as linguistics, may significantly improve the success of such mixed groups. These groupings can address pressing issues such as appropriate choices of assumptions, biological correctness, and applicability to bioengineering issues such as the role of bioelectrical signaling, interrelationships, scaling challenges, and tissue heterogeneity. Multi-scale modeling has the potential to reach the goal and connect those discrete areas, but it need be developed in a standard and well documented way so that it can be used by people without in depth backgrounds.

Need for methods to design, identify, characterize, store, and assure the quality of advanced biomanufacturing processes for diverse applications. Ideally, one would have a top-down design for the final assembly of biomaterials, including geometry, the specific interaction among cells, and the input/output behavior of cells and entire aggregates. Ultimately, the methods employed will need to specify the three dimensional spatial organization and help understand how it develops over time in terms of the mechanical, chemical and electrical properties of the system. When compiled this language would specify a number of physical interactions and processes necessary to achieve the goal. This would be further processed into a series of abstract physical implementations with known manufacturing processes for biomaterials scaffolds and cellular surface properties and cellular processes. Finally, this would be transformed into a series of molecular, genetic, cellular and material manipulations that could be carried out by a manufacturing process. The manufacturing process would be based on a series of standard primitives for these processes. Standard primitives—the biological and material functions that can be engineered and the manufacturing methods that implement them (additive manufacture, self-assembly methods, etc.) would have to be sufficiently characterized for predictable function (**Figures 9, 10, 11**). Predictable engineering includes the tools for directing and predicting the manufacturing process of these systems and the materials that allow in-situ real-time assessment of their progression and failures.

Recommendation: While there are emerging exemplars of modular materials and biological components that can be used to construct a limited diversity of applications, a more diverse set of functional systems for operation in more environments, with more actuation modalities, and better designed for interoperation are necessary. The creation of a computationally accessible knowledgebase of these primitives and their characterization is necessary to support a scalable computer-aided design and manufacturing framework. This leads to many needs:

- biosynthetic systems for cellular production and controlled secretion of structured biopolymers that form external structures and organize interactions at a high level.
- modular molecular elements of these biopolymers that predictable form self-organized supermolecular structures with known compatibilities with different environments.
- cellular sensors of electrical, mechanical and chemical signals that can affect cell and aggregate behavior.
- precision manufacturing for protein design, genetic encoding of functions in cells, biomaterial design, cellular printing into microniches that support aggregate development, and packaging for preservation. This is related to characterization and computer-aided design below.

An example target the system is one that should support integrated design system for a set of starting cells to produce surface proteins that direct cell-cell interactions sufficient to drive self-organization of diverse cell types starting from a bioprinted initial condition into a 3-D layered organization with organized activity from top-to-bottom and side-to-side. Also, to do this reproducibly and scalably such that hundreds of aggregates of defined size and composition can be produced quickly. A targeted multidisciplinary investigation of cell-material/microstructure interface to identify the key parameters for the design of the microstructure for desired cell generated biopolymer. Such investigation should involve a combination of theory and experiments.

Need for characterization and modeling of manufacturing processes: Physical components and manufacturing process reliability.

Recommendation: standards for measurement of primitive manufacturing elements need to be generated and to be sufficient to parameterize models that allow predictable manufacturing. The needs include:

- biophysical and statistical models that drive characterization of behavior of elements such as modular proteins in different contexts.
- synthetic niches to prototype and characterize these elements and their assemblies. For example, simulations of natural “organ” environments.
- knowledge-management systems that capture information about these elements and models.

Lack of tools for studying the bioelectric components of processes. While knowledge of action potentials is growing, understanding of the role of stable electrical states of non-excitabile cells is in its infancy. Currently we lack reporters, both exogenous and genetically encoded, that are designed for long term monitoring of, for example, membrane potential. This severely hampers our ability to understand and exploit these powerful signals despite abundant evidence of their power to control myriad cell processes, such as secretion, that are directly relevant to bioengineering.

Recommendation: Design and production of electrical-state reporters and controllers for long-term in situ use in non-excitabile cells. The former will provide critical information on both the health and activity of cells used in bioengineering and thus would address all four of the above described gaps in our knowledge. The latter will allow control of cell activities in new ways that are both simple and scalable. Preliminary evidence suggests that for certain cellular events bioelectrical control can obviate the need for complex protocols and cocktails of reagents, thus it could represent important cost-savings and improved efficiency.

There are no methods for studying phenomena specific to the *in situ* in vivo environment.

Recommendation: Design new sets of tools that allow diverse set of measurements *in vivo* non-invasively.

There is no common language allowing the interdisciplinary work that is vital to the success of bioengineering.

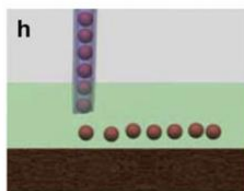
Recommendation: Design interdisciplinary education for graduate students, postdocs and faculty from diverse disciplines such that they can communicate with each other scientifically using common terminologies and similar basic principles.

There are no engineering approaches to design microenvironments for a given set of cells and cell clusters that result in a manufacturable polymer, or a live hybrid polymer with desired functionalities.

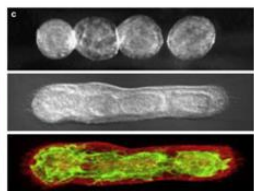
Recommendation: Fundamental investigations of cell-material interfaces through a multidisciplinary effort with expertise from materials science, engineering, biophysics and biology with a combination of theory and experiments. Such studies will lead to design parameters that quantify the interactions between cells and the microenvironment, as well as provide desirable design functionalities of the biomaterial such as optimal elastic modulus and extensibility.

Application in tissue patterning and formation

- Spheroid as building block for complex tissue formation



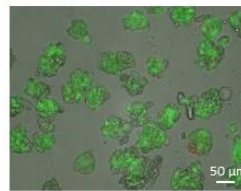
Bioprinting of living cell spheroids



Tissue fusion of vascular tissue spheroids placed in collagen type 1 hydrogel

Vladimir Mironov *et al.* Biomaterials (2009)

- Optimal microenvironment for cell clusters and ECM



Non-stained area in spheroids indicates cartilage-derived matrix powder, allowing microfunctionalizing of cells before loading into a macro scaffold.

- High-throughput screening

Flow cytometry can be integrated with high-throughput spheroid generation system to allow generation and selection of spheroids with desired expression of fluorescent markers

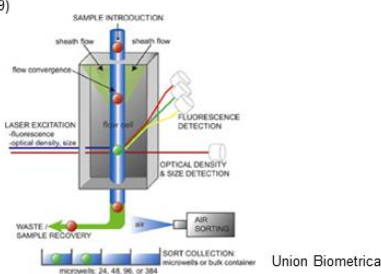
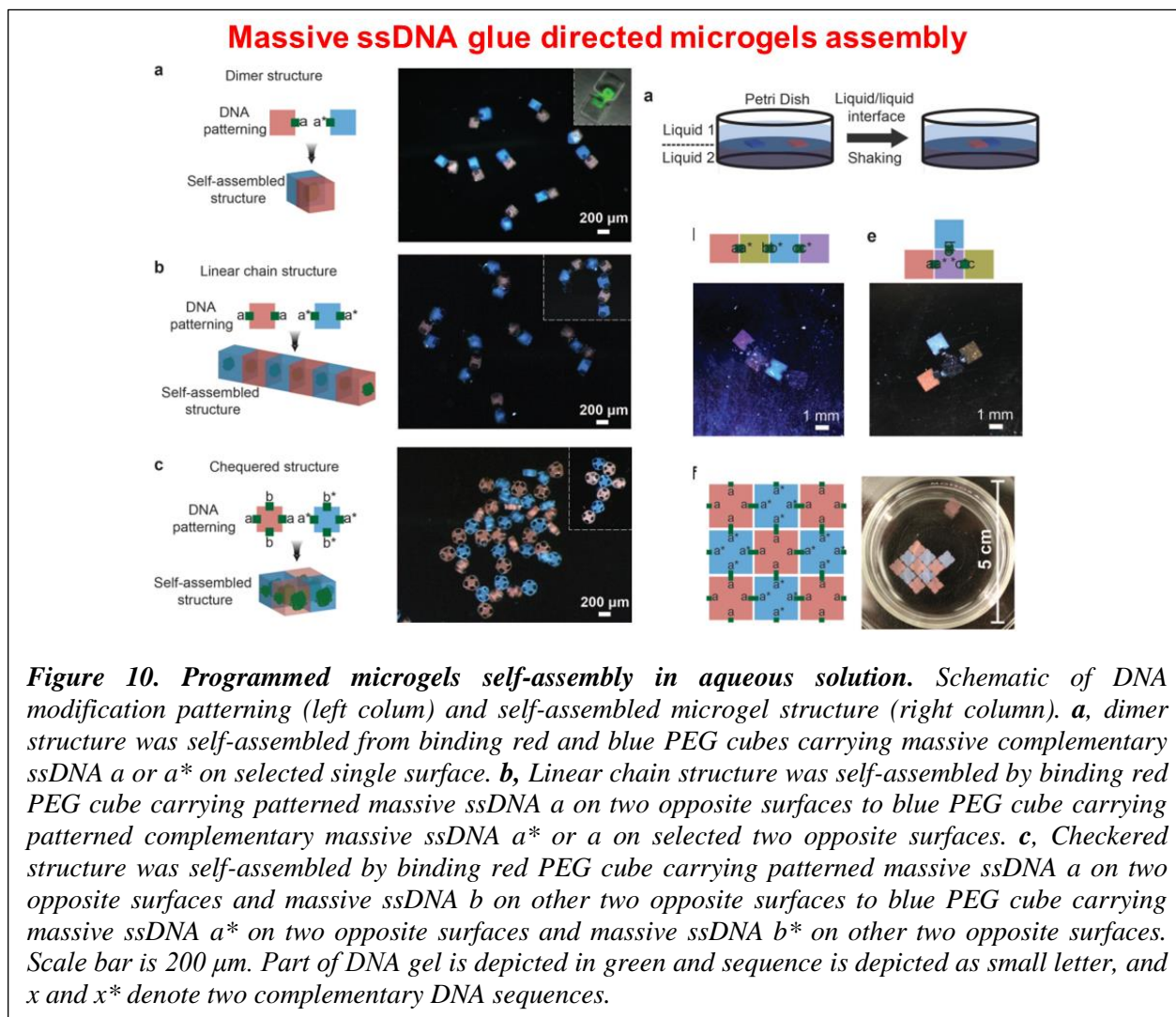


Figure 9. Sample techniques used to organize cells and tissues and to automate approaches.

Modeling and Simulation

There is lack of knowledge and physical models for cell-microenvironmental interactions *in silico*.

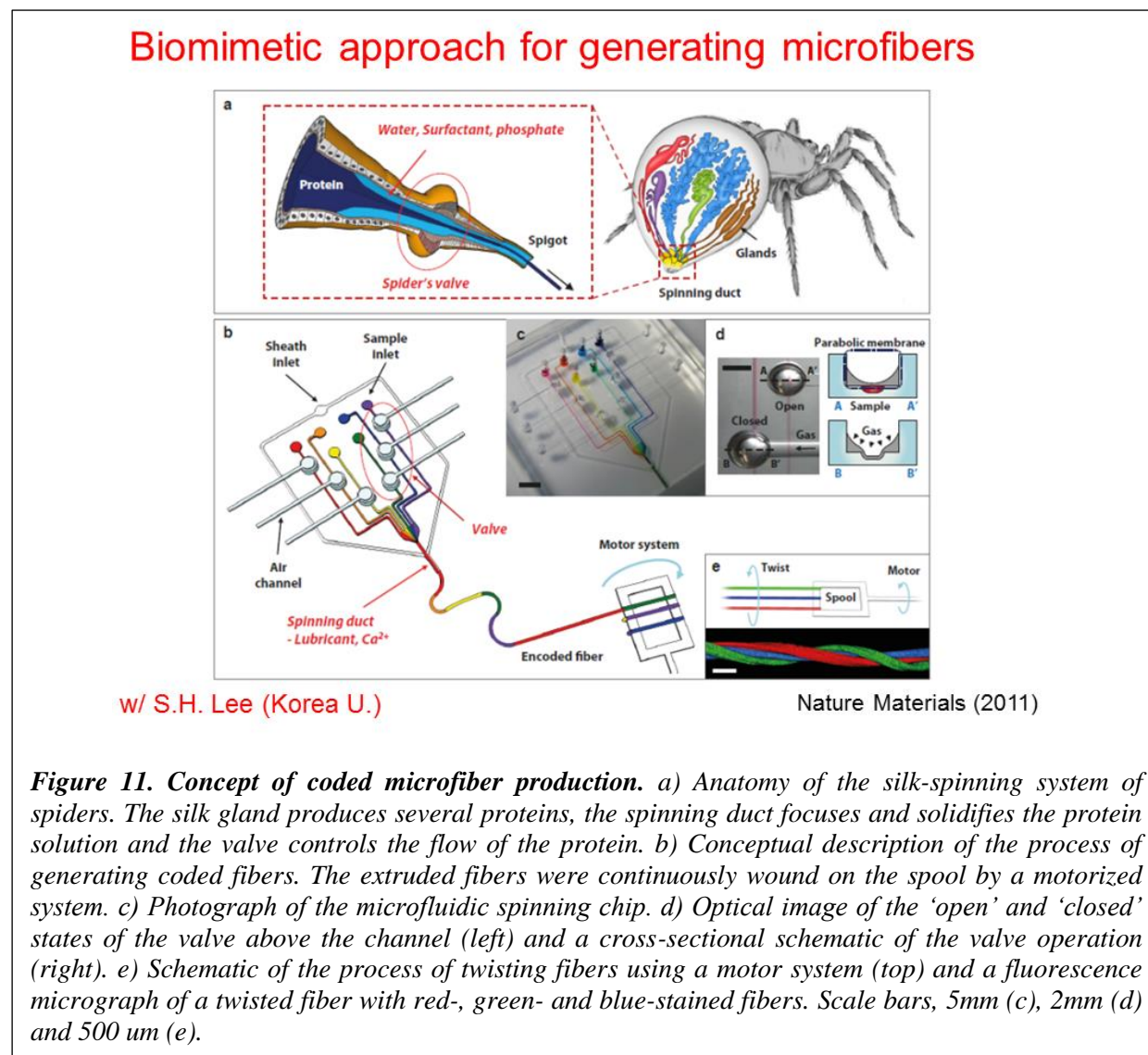
Recommendation: We need to consider the function of biopolymers by including their working circumstance instead of isolated systems. We need to include the effect of chemical environment (pH, temperature, ionic conditions, etc.) on the material functions into consideration. Indeed, this fact makes the material function, such as strength and degradation rate, no longer an intrinsic property of the building blocks *per se*. This method enables us to consider the interplay between the biomaterial and the environment in a dynamic way and the result will be helpful for the life cycle design of biomaterials.



There are few frameworks for transferring the knowledge from modeling and simulations to manufacturing of functional cellular and viral assemblies.

Recommendation: Development of integrated, scalable, and open, computer-aided design and manufacture and high-throughput screening technologies backed by the proper information systems to learn from failures and successes. The key is a rapid prototyping and screening infrastructure to support

this. Another idea is to connect experiments with bottom-up modeling and simulation to optimize the sequence of proteins, assembly conditions and process of their assembly. The knowledge is difficult to obtain from top-down studies but critical for advanced biomanufacturing.



Art of the Possible

- Novel highly functional biopolymers
- In-situ real-time monitoring and tracking
- Tunable functions of biopolymers according to design
- Drop-in tissues for therapy and products
- Novel biohybrid self-healing and sensing materials for both environmental and health applications
- Predictable and controllable life cycle of biopolymers
- Novel cell-based diagnostics
- Structural biosynthetic factories

- New materials for energy storage and generation (e.g., biobatteries with relevant output)
- Both simplification of process and scalability of production based on refinements of existing protocols and novel methods
- Production of viral materials for energy storage and energy generation
- Generation of bacterial assemblies for organized chemical factories, biofilms for material protection (oil well pipelines, ship hulls), sensors, replacement tissues, and self-healing materials

Regulatory and Cost Issues

- Deployment of cells/viruses and cell/virus aggregates into living tissues for applications to health and the environment still have many complex regulatory hurdles to overcome. The issues of biocompatibility, quality control, and long-term safety must be addressed.
- One issue that is of great relevance is the cost and scalability of cell-derived biopolymers. Due to the need to use cell bioreactors and biologically-derived systems, the processes for generating these materials are inherently slow. To enable translation of such products it is important to minimize batch-to-batch variability. Furthermore, it is important to address long processing, low yield and purification limitations.
- Infrastructure for manufacture requires sophisticated computation, instrumentation and automation. It is difficult for single labs to support such an infrastructure. Formation of biomanufacturing foundries that can be used by a range of laboratories will help advance the field. This will aid in establishing collaborations, informational interchange and standards as well. They would also form the basis of effective research centers. New models for handling intellectual properties would also be needed.

What are the needs for training?

- Training in theory and computation for modeling, theoretical biophysics, and mathematical biology to provide the foundation to understand the mechanisms mediating cell-microenvironment interactions. The theoretical framework to predict how cells behave in cell-cell and cell-substrate interactions ranges from rudimentary to non-existent. For instance, recombinant technology can generate a diverse range of biopolymers, but the prediction between DNA sequence and resultant biopolymer characteristics remains poor. Computational materials science can begin to predict structure-property relationship, but predicting how cells interact with biomaterials is an uncharted territory. Effort must be made to develop a theoretical framework of understanding how cells and viruses interact with their microenvironment. Multi-scale modeling of a dynamic environment would be a starting point.
- Training in biomanufacturing concepts and practices. To advance the field of biomanufacturing, a subset of the next generation of students and researchers must be conversant at the interface of biomaterials/biomedical engineering and manufacturing engineering. However, biomanufacturing is an evolving field and ill-defined. Furthermore, not all engineering schools offer manufacturing courses. Formal course work for students engaged in biomanufacturing research will be very limited in the near future. A remedy might be integrating workshop and internship opportunities into graduate training. This can take the form of in-depth summer workshops delivered by practicing professionals in manufacturing and summer internships in companies engaged in manufacturing.
- Training in automation technologies, systems engineering, and techno-economic analysis.
- Training in statistics and machine learning will be key as we transition into big data and reliability testing.
- Training in systems engineering, manufacturing processes, and techno-economic analysis

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Session 3 - Tissue/Organ Approaches

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State of the Art

The development of multicellular constructs including tissues and organoids will be critical in the future of advanced manufacturing in areas ranging from new tissues, environmental detection, synthetic foods, and personalized medicine. To build these multicellular constructs, manufacturing will be a critical component through research in engineering and science, which in the future will result in new industries and job creation.

One of the major highly promising directions in this area is in printing technologies, which are derived from various automated deposition schemes. Three-dimensional (3D) organ printing technology shows great promise as a viable option for creating a complex, composite tissue construct, which would be applicable in a diversity of areas including designed to regenerate or replace a damaged tissue/organ, use as a tissue diagnostic for environmental toxins or in building synthetic foods. These printing methods can precisely place cell-encapsulating hydrogels in a layer-by-layer fashion, replicating the complex 3D structure of tissues or organs of interest. New approaches such as integrated organ printing that can concurrently print synthetic biodegradable polymers and cell-laden hydrogels in a single tissue construct with applicable size, structure, and mechanical strength necessary.

Various technologies have been developed to print cells and manipulate them in small volumes (**Figure 12**). These technologies can be classified as nozzle-free and nozzle-based technologies. Some of examples of nozzle-based bioprinting technologies include: inkjet, piezo-jet, valve-based, and extrusion-based printing methods. These systems involve a droplet or a jet leaving a nozzle that encapsulates droplets and have been reported to print live cells and pattern proteins. Examples of nozzle-free technologies are laser printing and acoustic bioprinting. Laser printing involves a light beam controlling the locations of deposited cells precisely. Acoustic printing involves focusing acoustic waves to an open reservoir to generate droplets by breaking surface tension.

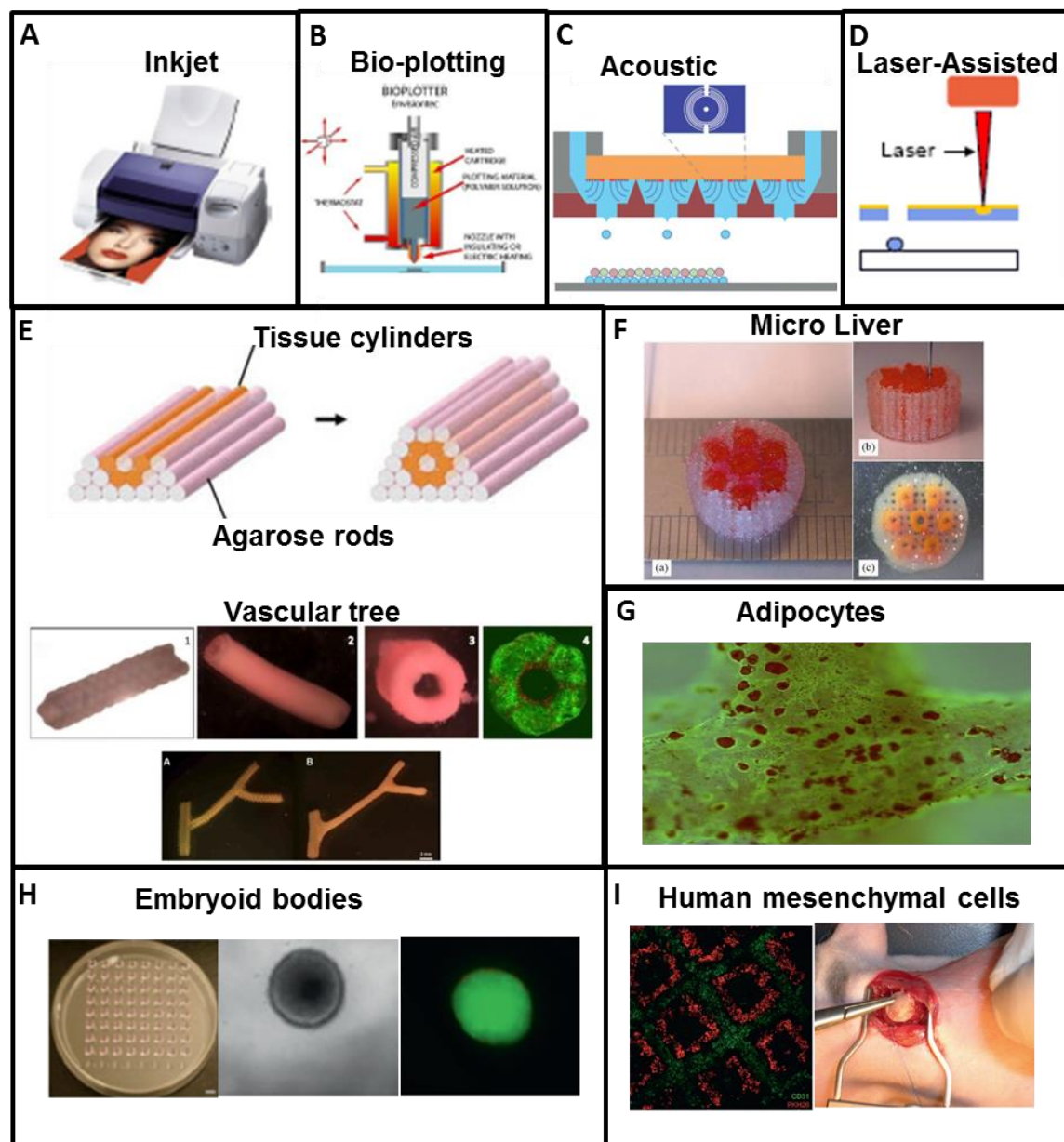
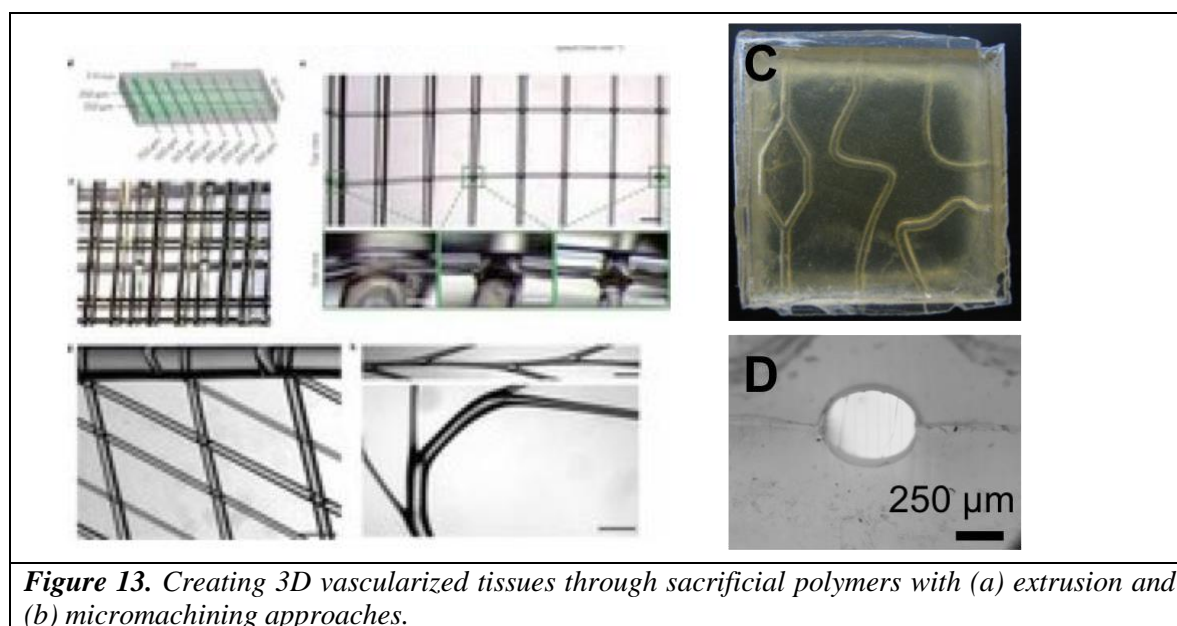


Figure 12. Bioprinting technologies and applications. Upper row: (A-D) Various bioprinting technologies are shown: Thermal and piezoelectric ink-jet printing, bioplotting, acoustic bioprinting, laser-assisted printing (top row). (e) Tissue cylinders were printed forming a 3-D vascular tree format. (F-G) Bioprinting has been used to print microscale tissue constructs using various cell-types. (H) Bioprinted embryoid bodies enable uniformity in size, cellular position and high throughput compared to manual methods. (I) Human mesenchymal cells were printed by laser induced forward transfer method and separated grid patterned HUVECs. The bioprinted patch was implemented in vivo.

These multicellular constructs can be assembled from cells as the building blocks such as with bioprinters. These present techniques allow also the incorporation of DNA and proteins and other molecular entities. The resulting tissue structures have been built up to 4 mm in thickness, which have been fabricated and implanted into animal models (Figure 1). Fully functional skin has been produced

using extrusion bioprinting. Bioprinted tissue arrays have been manufactured for drug testing. Biologics can be patterned in 2D and 3D arrays with the use of lasers and cell printers. For example, patches encapsulating human mesenchymal stem cells have been implemented in animal models. Additionally, 3D in vitro cancer model tissue constructs have been printed. These printed constructs can be used as model systems to mimic the complex native microenvironment of tissues as well as cancer models.

Another approach is developing highly sophisticated and varied scaffold structures that can be implemented to develop 3D tissues (**Figure 13**). This may enable complex vascularized and innervated tissues in the future. For example to manufacture tissue like systems with vascular conduits in 3D, fabricated sacrificial polymer layers can enable conduits to be created. These fabricated polymers can be created through approaches like extruding or micromachining, which can create non-planar features at a micrometer scale. These techniques enabled the fabrication of 3D structures that are derived from more conventional manufacturing processes that have been used in the steel industry for decades.



These patterning and assembly approaches are well positioned to be integrated with advanced manufacturing in the future to build many novel areas resulting in scientific advances, new companies, and job creation (**Figure 14**).

Gaps/Barriers

Gaps exist at different levels ranging from lack of fundamental design rules to developing novel computer code to run 3D printing machinery.

Incorporating developmental biology principles into tissue and organ engineering - Engineered biological constructs are fabricated, stored, and eventually deployed in environments different from the "natural" environment they are intended to reside. The biology of these constructs in these new environments needs to be established.

Cell to cell interactions need to be studied in multiplexed systems - To fabricate complex constructs and products involves combining multiple cell types in complex structures. It is more efficient and often necessary for the cells to interact with each other and develop, self-assemble, into the final structure.

Lack of database and “comprehensive theory” to guide structure building and formation - A theoretical basis for general tissue/organ building does not currently exist. A data base for the printing of different types of cells, interactions between different types of cells, culturing conditions, etc., is a necessary prerequisite for establishing a theoretical basis and guideline for further development in the field.

There is no understanding in the field of what tolerances in building 3D structures are acceptable - For further advancement of the engineering aspects 3D tissue printing a better understanding of the tolerances is needed in building 3D structures. While 3D fabrication of solid objects can occur with submicron tolerances, it may not be necessary to engineer tissues with those, as biological self-assembly is often occurring at those length scales. There is agreement that self-assembly does not occur at length scales in the mm-cm ranges within the appropriate time frame.

The definition of scale-up is not clear - “Scale-up” in production usually is perceived in terms of volume. In the case of 3D printing, “scale-up” may include an increase in the quantity produced, but biological factors are of equal importance such as an increase in number and types of cells printed. For non-planar (three dimensional) structures, an increase in volume while decreasing surface area presents critical challenges as the inner cells lose access to the external medium. Scale-up would necessitate the development of truly large-scale cell culture techniques for the production of cells to print organ-size multicellular constructs. Such techniques do not yet exist.

The integration with host tissue has been recognized as critical but has not been studied systematically - The advantage of tissue/organ printing over other tissue engineering technologies is that vascular conduits can be constructed within the scaffold/tissue layers as they are built up. Vascularization with host tissue is seen as a critical step in assuring success. Ex-vivo models of neo-vascularization have been developed and can be exploited in a systematic study of printed tissue/host vascularization.

New printable biomaterials and bioreactors need to be developed - Nature is replete with biologically produced or precipitated materials, and synthetic biology is producing even more. As the technology matures and many new applications are anticipated, new materials will need to be identified or engineered to meet these needs. Bioreactor technology today is focused on a few microbial species (e.g., yeasts, *E. coli*) but as new production cells are needed, new bioreactors will need to be developed.

Multicellular structures can be produced, but the products do not have the necessary cues needed the fully biological - A barrier to building multicellular structures that are functional is the lack of understanding what necessary cues that needs to be provided by scaffold or matrix. Development of *in vitro* model systems that allow testing the effects of single and combinatorial effects of factors and/or signaling molecules on the function of tissue constructs is needed. Tools developed for cell printing and/or deposition are seen as playing an essential role in this development.

Computer control of equipment to activate manufacturing devices is limited - To achieve manufacturing, high throughputs, precision, speed, and repeatability are critical. This can only be achieved through automation. Depending on the spatial scale of the final product, the process will need to scale up from laboratory scale. The monitoring and care of products post manufacturing are also important factors for

commercialization. Computerized equipment is central to all these processes, which is not available currently.

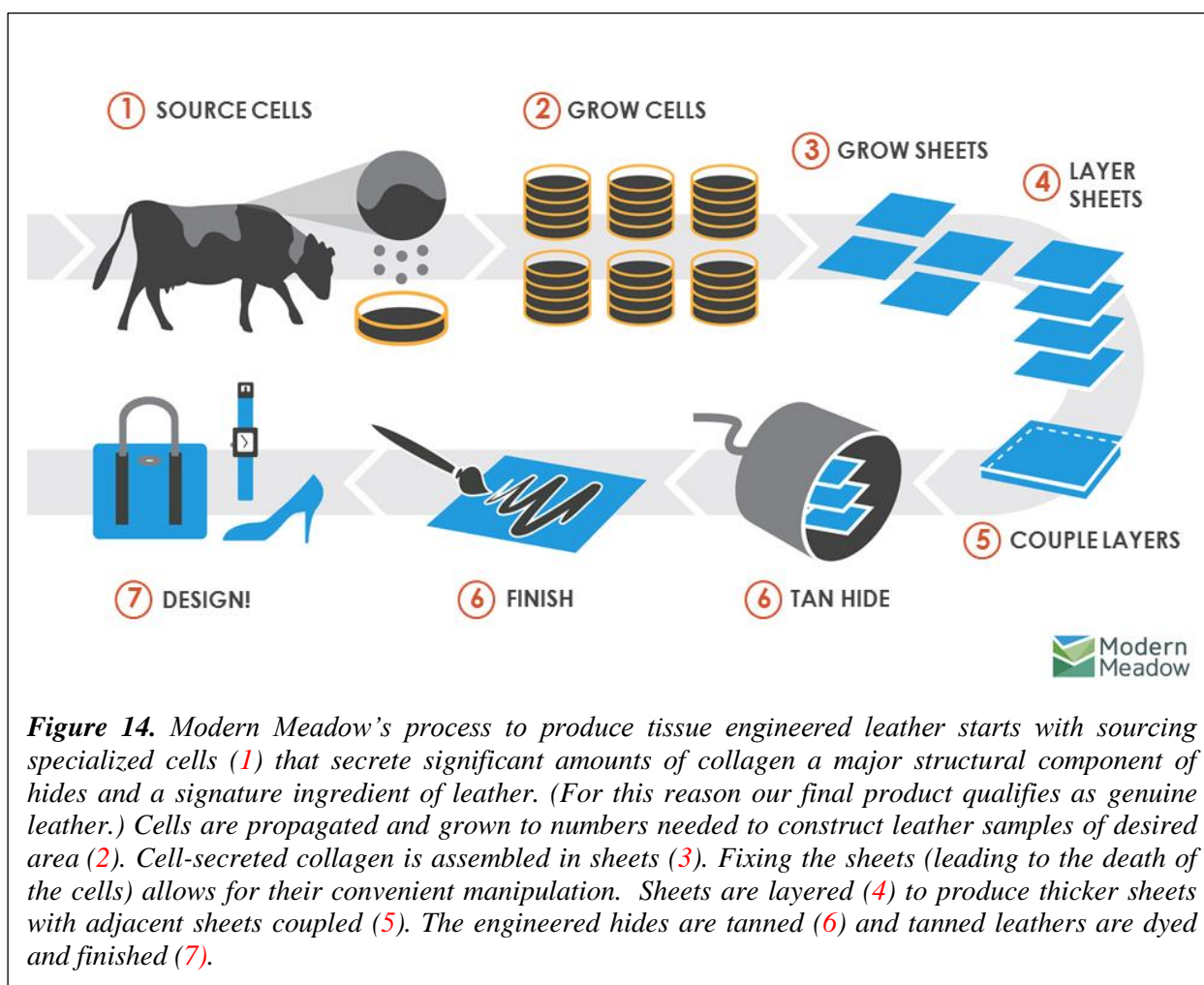


Figure 14. Modern Meadow's process to produce tissue engineered leather starts with sourcing specialized cells (1) that secrete significant amounts of collagen a major structural component of hides and a signature ingredient of leather. (For this reason our final product qualifies as genuine leather.) Cells are propagated and grown to numbers needed to construct leather samples of desired area (2). Cell-secreted collagen is assembled in sheets (3). Fixing the sheets (leading to the death of the cells) allows for their convenient manipulation. Sheets are layered (4) to produce thicker sheets with adjacent sheets coupled (5). The engineered hides are tanned (6) and tanned leathers are dyed and finished (7).

Art of the Possible

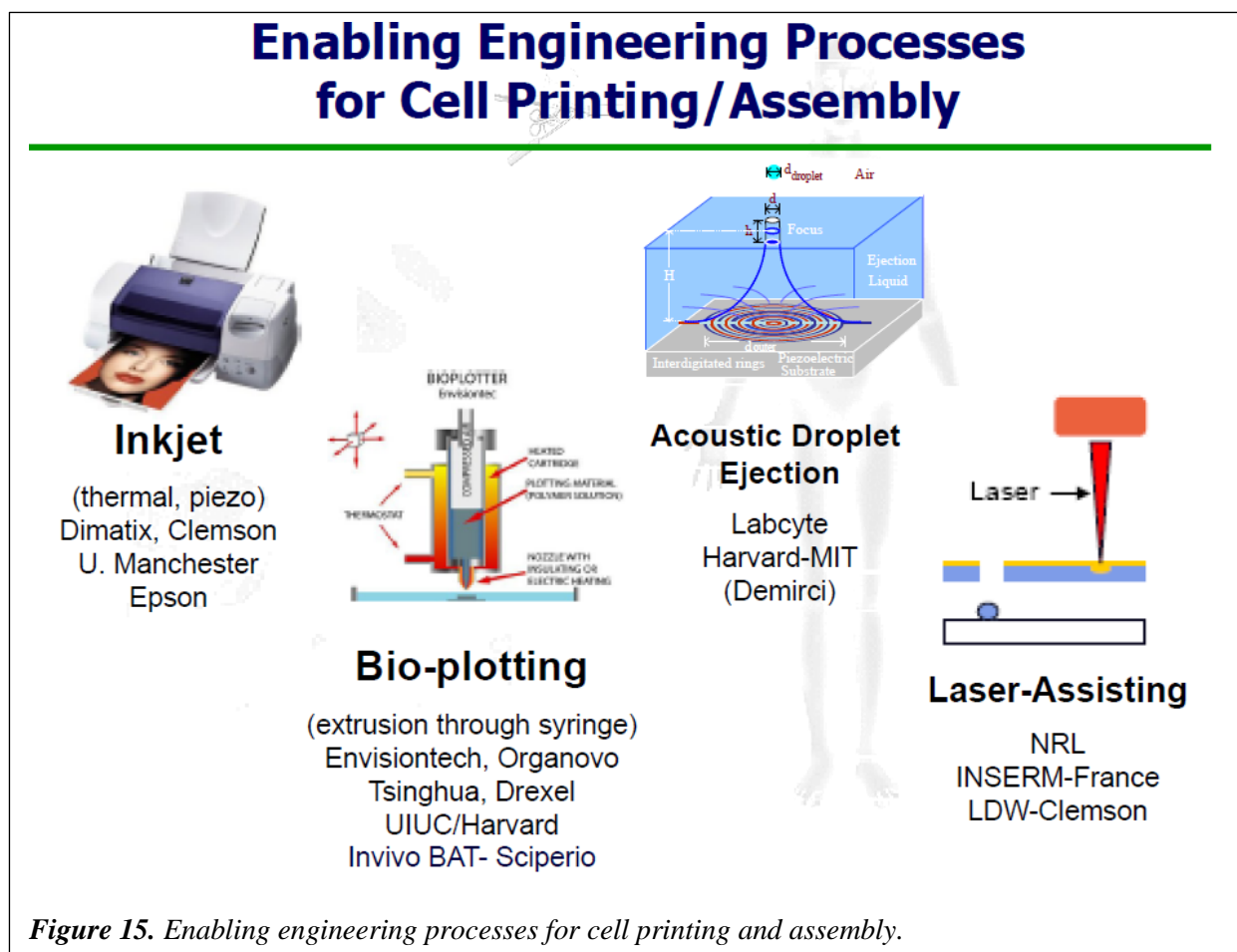
Present knowledge and technologies in tissue and organ manufacturing (Figure 15) provide realistic blueprints that the following will be possible to accomplish in the near future.

In regenerative medicine

- Several similar approaches have been suggested to overcome the major hurdle of vascularization needed to fabricate extended solid tissue and organs. Such structures cannot be kept alive by relying solely on the diffusive transport of nutrient to all cells. This can be accomplished by the active transport of the nutrients through a network of branching conduits (the vasculature) that assures that no cell is farther than 2-300 microns from this supply mechanism.
- All the suggested approaches are based on “sacrificial conduit networks”, indicating that the solution to this problem is converging. A sacrificial network is a blueprint of branching vasculature fabricated

from a material that serves as a temporary mold. The cellular structure is constructed around this structure and, subsequently the mold is sacrificed that is removed (e.g. by variation of the temperature). The remaining network of hollow conduits is flushed with endothelial cells, which eventually seed the lumens of these conduits, providing the protective barrier akin to that in a natural vascular network.

- Sacrificial network molds have been fabricated either with 3D printers or micromachining tools (with linear features of 10-100 microns. Once printed multicellular structures, engineered tissues are supplied with such engineered vasculature they will be possible to maintain in vitro until they are matured to the point that they can be used for implantation. This program will allow us to fabricate off-the-shelf tissues (and eventually organs).



In manufacturing

- The working group feels biomimetic manufacturing will become a new paradigm. New materials and processes will be developed based on the capabilities of the living material, in particular the cells. We envisage several possibilities at present and believe the range of future possibilities is very wide.
- Cell-produced materials with unique properties, such as bone, could be used as construction material. Cultured leather could be used in the fashion, shoe and auto-industry. Such applications will lead to considerable savings in resources (energy, water, land) and eliminate adverse present industrial practices (e.g. toxicity in leather production).

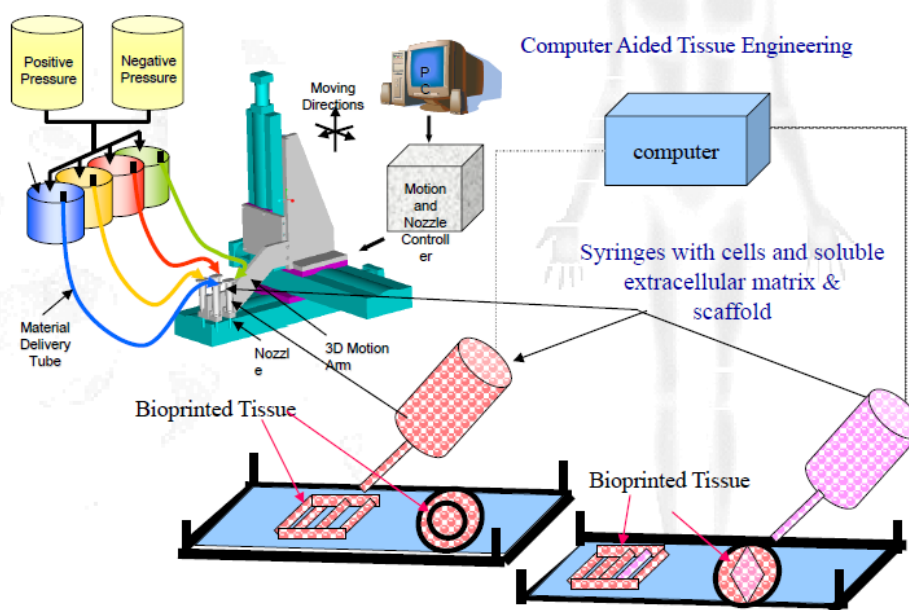
- Harnessing nature's skills to fabricate tissues will allow producing food for use in constrained environments (e.g. space ships, battle ships). Learning how to employ cellular machines (e.g. molecular motors; acto-myosin contractile system) at the tissue level, will lead to our ability to perform specific tasks across scales such as miniaturizing devices for medicine, or performing work with engineered muscle.
- Further automation of the entire tissue and organ engineering process will lead to more efficient fabrication and our ability to produce these structures "on-site" as needed. Ultimately we envisage that patients will walk into specialized facilities, shed their dysfunctional organs and have ones "made to measure".

In disease modeling and drug testing

- Cell printing permits the generation of three-dimensional *in vitro* tissue models for probing basic biological insights into cells and tissues as well as understanding human disease processes.
- Among the applications are three-dimensional *in vitro* tissue analogs that mimic different cancer tissues to elicit mechanistic information. For example, microprinting of cancer cells patterned with fibroblasts and various angiogenic factors can simulate some of the hallmark features of invasion and metastasis seen in cancer patients. A micro-fluidic device housing three-dimensional biofabrication tissue constructs can be developed to enable manipulation of these cells in a three-dimensional microenvironment to help explain the fundamental biological processes of cell-cell and cell-matrix signaling and interactions, as well as allowing for environmental toxin screening.
- Replicating cell and microenvironment in 3D is critical in understanding the physiology and pathology of human tissue conditions. Three dimensional tissue models permit understanding of cell and tissue behaviors in response to external stimuli. As such, use of this system may recapitulate an individual's medical condition in vitro, which would allow for the development of personalized therapy.
- Cell/Tissue/Organ-on-a-chip technology provides a vital tool in developing disease models and drug testing. These microchip devices would not only mimic the cell microenvironmental characteristics in vivo, but also integrate the dynamic cell culture and high-throughput analysis together, mimicking specific organ activities, mechanics, biophysical responses and functions in vitro. With controlled fluid properties in microchannels, one can simulate the physiological conditions for tissue/organ growth in the chip.
- 3D cell printing technique, based on bio-additive manufacturing technology, can directly print cells with appropriate delivery medium to build 3D tissue constructs layer by layer (**Figure 16**). This approach allows the assembly of heterogeneous tissue structure to mimic in vivo tissue model, as well as the possibility of fabricating 3D in vitro tissue model with large-scale, high throughput and high cell density. And it can be used to print specific cells or biomaterials at a specific spatial location in the channels of microfluidic and reduce the process of cell co-culture in microfluidics and the time.

Bioprinting Micro Liver Organ for NASA Pharmacokinetic Study

(NSAS-USRA-09940-008)



Chang and Sun, Tissue Engineering, 2007 & 2008

Figure 16. Schematic of bioprinting dual micro-organ systems.

Modeling and Simulation

To understand the complex interactions that occur in building tissue and organ like systems, it is critical to understand their multiscale integrated biological responses. One approach to this is to use developmental biology as a model system as it is a naturally occurring multiscale (molecular to cellular to multicellular) system. Models will be built examining these systems taking into considering a multitude of factors including biochemical, scaffolding, mechanical, electrical, etc. These models have to cross multiple scales yet these scales and multitude of interactions will cause challenges to occur. Models to understand these interactions and then use these findings to predict future integrated tissue responses need to be implemented such as those with complexity, coarse graining, multiscale and many others will have to be applied and adapted to probe these biological systems.

More specifically in printing, controlling the cellular microenvironment and understanding how we can model and understand controlled release of molecules post-printing is a significant area of interest. For example, there is a need for multi-scale modeling for understanding the distribution of cells in spatial and temporal domains. In recent years, single cell studies are gaining importance as encapsulating single to few cells has been applied to broad applications in studying heterogeneity in cancer, immune response with broad applications in single cell studies. Enhancing our understanding of how cells are encapsulated

in droplets is one of the major theoretical gaps that will allow us to build such technologies. There needs to be both probabilistic and experimental methods that are broad and applicable to various methods to understand this process.

Regulatory and Cost Issues

Regulations for printed tissues and organ structures are not well defined. Manufacturing devices and their products are looked at on a case by case basis by the FDA.

Training and Education

Workforce development within the focus group of organ and tissue development is envisioned to occur at levels from associate degrees to PhD and postdoctoral levels. The closest existing programs are the degrees in BME. Graduates typically would need on-job training. While this is expected, current curricula in BME typically do not incorporate critical elements such as developmental biology or automation. Therefore, we propose the following education and training innovations:

- Establishing concentrations in degree programs (BS through PhD) focusing on advanced biomanufacturing,
- Fostering close partnerships with industry and clinics (e.g., through internship programs)
- Establish educational objectives and program evaluations,
- Outreach to community colleges to align curricula with concentrations in biomanufacturing,
- Foster a culture of entrepreneurship, by incorporating entrepreneurship courses into the formal education, mentoring and workshops
- Disseminate innovative curricula in academia and professional societies.

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Session 4 - Aspects of Systems Integration in Biomanufacturing**Session Co-Chairs:**

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State of the Art

Strategies for Comprehensive Systems Integration are Largely Product-Specific. A key theme in systems integration is the diversity of the challenges (**Figure 17**). The specific challenges in systems integration are largely defined by the specific product. Other kinds of biomanufacturing strategies that are unilateral in terms of length scale and functionality can be leveraged to fabricate multiple diverse products. For example, molecular level approaches can be leveraged to produce a variety of proteins for use as structural materials, functional materials, or therapeutics. Other examples at the cellular and organ levels have been previously described as well. These technologies can be used to fabricate specific articles. However, systems integration is charged with the challenge of uniting these articles together in a way to produce a larger multiscale system. Systems integration will ideally start with the final product, identify the relevant technology or technologies, and then integrate them into a system that can ultimately be used to fabricate the original product in mind. Systems integration deals with the seamless melding of these technologies in order to generate robust, scalable, and economically viable biomanufacturing systems. There are many challenges in this process including the following:

- Identification of cross-over points in which the output of one discrete technology or process can serve as the input of another process.
- Uniting stakeholders and end-users in defining appropriate metrics both within length scales (molecular, cellular, and organ) and across length scales.

Systems Integration Challenges are Numerous and Multifactorial. Systems integration approaches can be parsed out to include multiple unique thrusts. Here we delineate a difference between process integration and systems-level design. Process integration is a key element of biomanufacturing. Process integration is defined as the ability to connect discrete unit operations of a broader process in a tractable manner. Strategies for process integration can be derived from those developed in chemical engineering. Systems-level design utilizes aspects of process integration for the goal of comprehensive systems design. Examples of systems-level design includes multiscale fabrication strategies for the integration of materials and cells with electronic devices including biosensors, electronic elements, and higher-level logic.

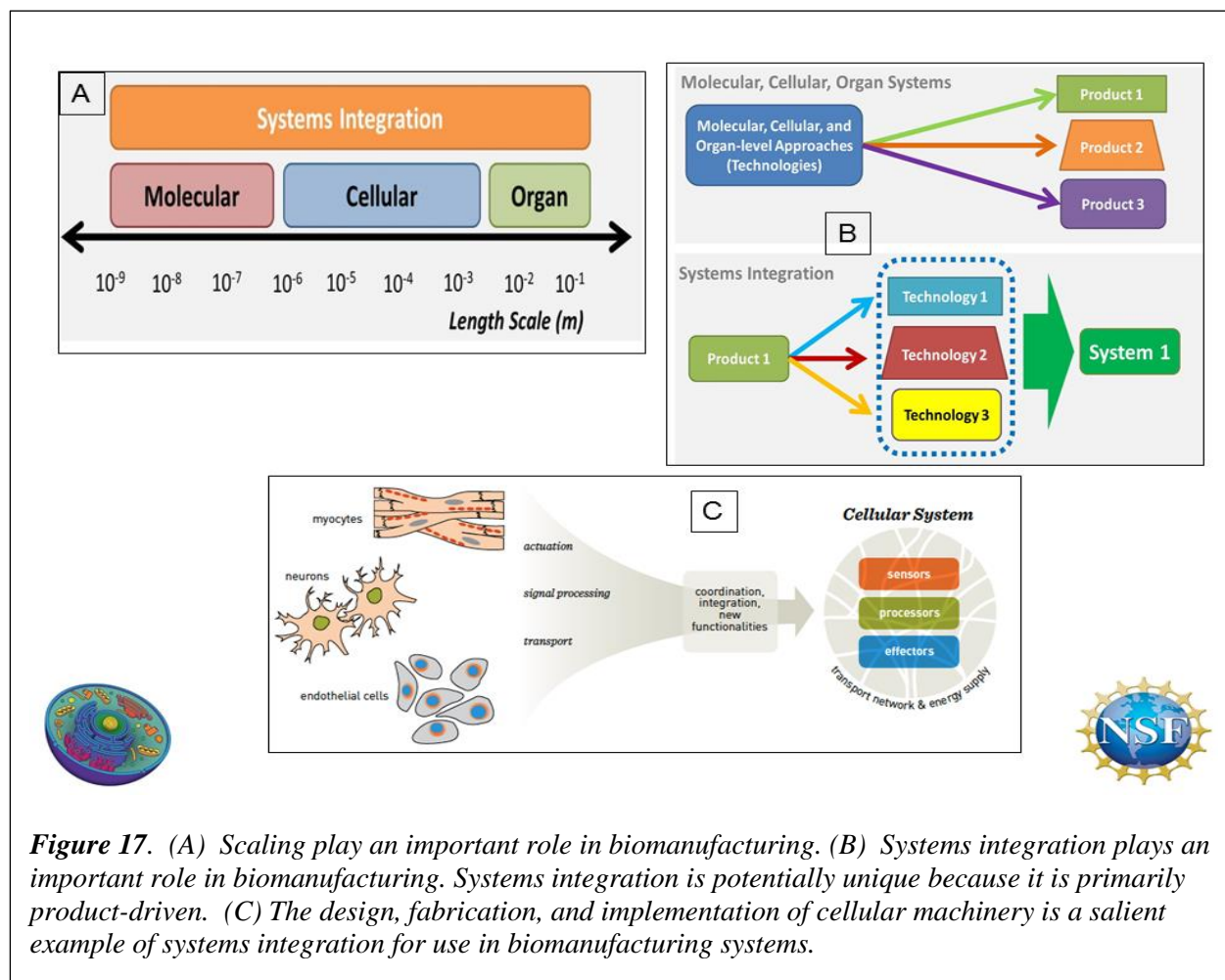


Figure 17. (A) Scaling play an important role in biomanufacturing. (B) Systems integration plays an important role in biomanufacturing. Systems integration is potentially unique because it is primarily product-driven. (C) The design, fabrication, and implementation of cellular machinery is a salient example of systems integration for use in biomanufacturing systems.

Gaps, Barriers

Many challenges and opportunities exist in the bio-manufacturing of biological and cellular systems. These could be categorized as fundamental biological issues and aspects, and issues that are more technology and manufacturing related.

Fundamental Aspects

- There is very limited understanding of cell-cell interactions and communications and our abilities to manipulate these interactions. How do cells respond to physical and spatial gradients and how these cues affect the autocrine and paracrine interactions? The complexity of these interactions increases dramatically as heterotypic cellular interactions are to be considered. Imaging, sensing, and modeling approaches for examining and understanding these interactions are very much needed.
- Characterizing and controlling the issues of consistency and variability of cells and biomolecules is another challenge. If cells or biomolecules are to be used for producing another product or if

cells are used to make cellular systems, the control of parameters describing the physical and chemical properties of cells will be very important for biomanufacturing.

- Co-differentiation of cells from embryo bodies and cell clusters is a challenge and an opportunity to produce different cell types at the same time so their interactions could be tailored would be very useful.
- There exists tremendous potential and unexplored potential for using cells from different kingdoms (animal, plants, insects, microorganisms). Extracting opportunities for biomanufacturing of chemical and biological product from plant, insect and bacterial cells could be very important. Similarly, use of cells across these species for the development of cellular machines could be very useful.
- Could mammalian cells be reprogrammed to operate at other temperatures except at 37°C, e.g. at room temperature?
- Prediction and control of emergent behavior of cellular networks is a grand challenge for biomanufacturing.
- Technologies for characterizing and measurement of various physical and chemical properties for cell-cell communications and cell-matrix interactions need to be developed. Approaches such as imaging, chemical probes, computation, etc. will need to be integrated for measured 4D interactions.
- Reliable vascularization continues to be an issue and a challenge and every living system and exchange of nutrients and wastes will be critical to long term operation of these systems

Art of the Possible

Examples of Initial Successes. There are many examples of initial successes in systems integration. These include many technologies, which have enjoyed a high degree of near-term commercial interest. These technologies can be best characterized by a clear, but difficult, path to commercialization. Examples of these technologies include the following recent advances which are admittedly at different technology readiness levels:

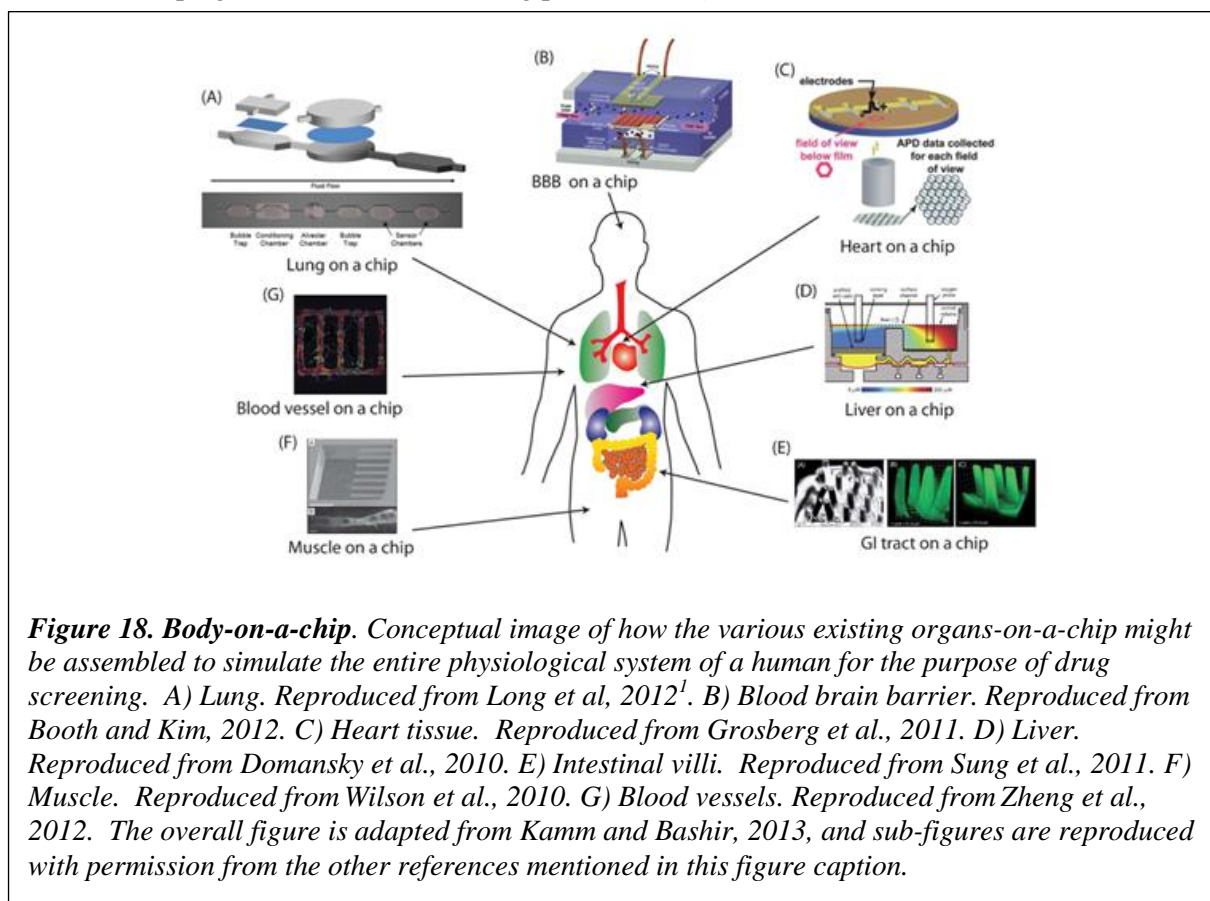
- The design and validation of processes for the synthesis, extraction, and purification of naturally occurring materials with unique bioelectronic functionality. These may be useful for applications in bio-inspired electronics and devices.
- Engineering cells to produce biofuels with high efficiency
- Bio-manufacturing of consumer products or defense applications
- Human on a chip
- Bio-manufacturing of ‘insect-like’ systems for sensing and response (environment, health, etc.)
- Bio-manufacturing of inorganic devices (photonic, electronics, materials)
- Design and fabrication of biotic devices such as brain-machine interfaces and retinal prosthetics.
- Biodegradable and environmentally-benign electronics.
- Biological prosthetic devices to integrate human tissues with external systems.

Examples of Long-Term Projects. Conversely, there are many examples of systems integration for biomanufacturing which carry more intrinsic risk. These directions are equally exciting and would benefit from sustained stable investment

- The design and synthesis of cellular machines for specific applications
- Fabricating materials and interfaces to enable “Hyper-organs” in which the window of function for natural tissue and organ structures can be expanded into supernatural realms. For example, ultrasonic hearing or infrared vision in implanted tissues.
- Design and bio-manufacturing of plants, wood, and other commodity materials.
- Closed-loop autologous systems for disease treatment
- Cell-based energy conversion/transduction systems
- Room temperature processing of mammalian cells
- Self-replication as a means for bio-manufacturing

Technology and Manufacturing

- The spatial-temporal control of cell behavior & function would need to be controlled for developing robust bio-manufacturing processes (**Figure 18**).



- The cell culture systems will need to be optimized for specific applications and specific market segment. The approaches might be modular and could be application specific versus core bio-manufacturing modules that are applicable across many applications.
- The process control and issues of variability are more important for cellular and biological materials and cells compared to synthetic or electronic manufacturing. Similarly the issues of biological product stability, preservation, storage, biocompatibility and toxicity are important and would need to be considered.
- The concept of Emergent or Adaptive biomanufacturing that where the assembly might be emergent or the final product might be emergent itself in the sense that it can continue to remodel in response to changing conditions.

- Bio-fabrication approaches cannot be considered high throughput yet. Cell printing and placement, laser based polymerization, etc. could be integrated with high speed roll to roll printing and other emerging biofabrication approaches could be integrated to realize new capabilities. This is also related to the balance between high throughput and low throughput processes for the appropriate applications.

Non-Technical Issues

A variety of non-technical and regulatory issues and barriers need to be addressed for increasing the impact and pervasiveness of the regulatory barriers. These include (i) developing standards for cell phenotypes and manufacturing of the modules, (ii) interdisciplinary language barriers, (iii) ethical issues related to bio-manufacturing and self-replication, and (iv) issues related to technology adoption, ease of use and functionality.

Modeling and Simulation

Modeling and simulation is a key aspect of systems integration. In the context of systems integration, computational models can be used to highlight some key aspects of biomanufacturing. Specifically, the following provocative questions would be interest to the biomanufacturing community.

- **Noise & Error in Biological Systems.** How much noise is too much noise? How can these definitions be addressed and modified for specific applications in systems at the different levels including molecular, cellular, and organ scale devices.
- **Signal Transduction.** How do we characterize noise propagation and information transfer in systems? How can figure of merits be translated to and from different aspects of the process.
- **Fault Tolerance and Failure Modes.** How can we model fault tolerance in biological systems? What role can failure mode analysis play? How can we model these processes?
- **Abstracting Standards in Molecules, Cells, and Organs.** Can we use modeling to clearly define engineering parameters in cells? For example, in polymeric systems, complex solutions can be abstracted into practical engineering parameters such as molecular weight, viscosity, etc. Can we recapitulate these values for cells and organs? Where can modeling help in this process?

Regulatory and Cost Issues

Advanced biomanufacturing follows the essential philosophy of product design and manufacturing process, except that the process could be more complicate. From regulatory perspective, it is always good to engage discussion with regulatory authorities and understand requirement depending on the area where the product will be used for. Some regulatory requirements should be even implemented into the product design and manufacturing process. If the product development involves biological components or studies in animals or human beings, ethic issues should be considered as well.

To drive down the cost of biomanufacturing, scalability and manufacturability should be incorporated as part of product design and manufacturing process design. Other traditional manufacturing considerations, such as quality control, quality assurance, and supply chain validation, apply to biomanufacturing as well, although they could cost more depending on the complexity of the system. As part of the manufacturing process, integration between biological modules and non-biological modules could incur higher cost due to compatibility, sterilization, and special packaging condition requirements. When delivering the final product to the end user, how to provide the product with longer shelf life, how to make shipping and storage more cost effective would add more value to the product.

Education and Training

Educational and training opportunities are an important component of systems integration. One of the key strategies in educational opportunities is to obtain immediate buy-in from stakeholders at the genesis of novel training programs. Presumably, the stakeholders of these programs will be private organizations and companies that will be able to hire the graduates that graduate from said training. In addition to overarching recommendations and directives, the interface between the academic and private sectors would be blended if additional investments are utilized such as formal externally-funded training programs and public-private partnerships. The following educational opportunities may be pursued:

- Dual degree programs in which experts from disparate backgrounds are trained in the complementary discipline. For example, educational programs could be used to fabricate biomaterials into useful electronic, photonic, and phononic structures. Such a program would lie at the interface of polymer science and microfabrication. In another example, process engineers could be trained in tandem with molecular biologists or geneticists.
- Interdisciplinary educational and training opportunities are likely essential. The key is to identify gaps in specific orthogonal disciplines followed by the creation of these programs.

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